Studies on Common Scab of Potato

by

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Submitted in fulfilment of the requirements
for the Degree of
Doctor of Philosophy

University of Tasmania
April, 2000

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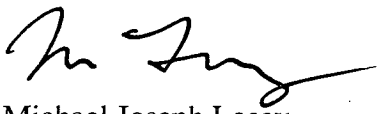

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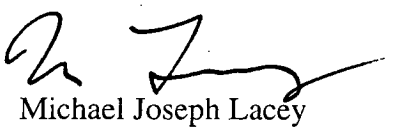

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Thesis abstract

This thesis includes studies on: 1) characteristics of streptomycete isolates from common scab lesions of potatoes collected from across the Tasmanian potato cropping region; 2) scab conduciveness of soils from that region and ; 3) effects of green manures on common scab of potato.

Most of 94 streptomycete isolates fitted into four groups based on morphological and physiological characteristics. Groups were: *Streptomyces scabies* (25 stains); *S. violaceusniger* (11); *Streptosporangium* spp. (3); and strains resembling *Streptomyces halstedii* (29). Some of the remaining 26 ungrouped strains resembled *S. scabies*. Most *S. scabies* strains could grow at pH 4.5.

Pathogenicity of strains was evaluated in four ways. In a potato disk assay, all *S. violaceusniger* strains, *S. scabies* reference pathogen strain #32 and five *S. scabies*-like ungrouped strains showed clear signs of pathogenicity. Media from oatmeal-broth (OMB) cultures of *S. scabies* and *S. violaceusniger* strains inhibited radish seedling growth, while *S. violaceusniger* strains 54/3 and 75/1-1 killed seedlings. Surface necrosis occurred within 24 hours of dipping potato minitubers in media from OMB cultures of *S. violaceusniger*. Three *S. scabies* strains (including #32) also produced darkening of lenticels, indicating necrotising potential. *S. violaceusniger* strains produced nigericin while strains 54/3 and 75/1-1 also produced geldanamycin, probably accounting for their virulence in pathogenicity assays. Thaxtomin A production by strain #32 was also confirmed.

Scab conduciveness of 36 Tasmanian potato cropping soils was compared in a glasshouse trial. Linear regressions showed no association between scab severity and any of 12 individual soil chemical properties. However, scab was much less likely below a threshold pH or when the concentration of exchangeable cations Ca^{2+}, Mg^{2+} and K^{+} (considered together as milliequivalents) were below a threshold value.

In glasshouse studies, green manures did not increase scabbing and in one pot trial a broad-bean manure reduced scab severity. Green

manures were associated with increased cellulase activity and electrical conductivity (EC) of soil in the absence of added fertiliser plus, in some trials, increased tuber yields. Added streptomycete antagonists did not noticeably effect scab severity.

In field trials at two sites, lupin or ryegrass green manures did not effect scab severity, while increased scab with an oat manure at one site was associated with increased soil EC. Increased scab with a canola green manure, evaluated at only one site, was associated with reduced soil microbial activity as assessed by FDA hydrolysis. At field sites, green manures did not affect tuber yield, soil cellulase activity or pH.

Acknowledgments

I would like to thank my supervisor Calum Wilson for his assistance during the course of this project and my industry supervisor Les Murdoch for his assistance with field aspects of the project.

I would also like to thank the following:

Margaret Stroman (dec.) of DPIWE for providing *Streptomyces* strains and the method for isolating further strains use in this study,

Brett Pemberton for providing soil samples from the 1995 DPIWE scab survey,

Noel Davies of the Central Science Laboratory, University of Tasmania for mass spectrometry,

Graham Rowbottom of the Central Science Laboratory, University of Tasmania for elemental determinations on vegetation samples,

David Ratkowsky of the School of Agricultural Science, University of Tasmania for advice on statistical analysis and for running the Taxon software for numerical taxonomy of the actinomycete strains,

Mike Creswell and Rex Sykes for allowing green manure field trials to be conducted on their properties,

Jo Dean for her help in establishing the field plots,

Chris Russell of Simplot and Sue Hinton of McCain Foods for help in finding field sites,

Barbara Fry of the Department of Plant Pathology, Cornell University, Ithaca, New York for the method used to test pathogenicity with minitubers,

Greg Luckman of the School of Agricultural Science, University of Tasmania for information on thaxtomin production by *Streptomyces scabies* strain #32.

Leigh Sparrow of TIAR and Richard Doyle of the School of Agricultural Science, University of Tasmania for advice on soil chemistry.

Chemical analysis of soil samples from the 1995 DPIWE scab survey and the soil used in the first green manures pot trial was carried out by Allison Laboratories, Hobart, Australia.

Financial support was received from an APA(I) scholarship provided by the Australian Research Council and industry contributions from McCain Foods (Australia) Pty. Ltd. and Simplot Australia Pty. Ltd.

List of Abbreviations

Media (See Appendix 1.)

CMCA	carboxy-methyl cellulose agar
GAT	glycerol asparagine tyrosine agar.
YME	yeast-malt extract agar
PYI	peptone yeast extract iron agar
OMB	oatmeal broth

Mass Spectrometry

APCI	atmospheric pressure chemical ionisation
EI	electron-impact ionisation
ESI	electrospray ionisation
LC-MS	liquid chromatography with mass spectrometry
M+	molecular ion
m/e	fragment per unit charge

Other

cfu	colony forming units
CMC	carboxymethyl cellulose
DPIWE	Department of Primary Industry, Water and Environment, Tasmania
EC	electrical conductivity
FDA	fluorescein diacetate
ISP	International Streptomyces Project
TIAR	Tasmanian Institute of Agricultural Research
TLC	thin layer chromatography

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1 Introduction

Common scab is a disease that affects potatoes (*Solanum tuberosum* L.) wherever they are grown throughout the world. It is characterised by corky lesions on the tuber surface, which may be superficial, raised or sunken. This scabbing is the result of infection of tubers by *Streptomyces* species, most commonly *S. scabies.*

Common scab does not usually have significant effects on plant vigour or tuber yields, the main effect being economic, due to downgrading of tubers from fresh market to processing quality. Where lesions are deep pitted additional processing costs are incurred in extra peeling. A 1991 survey of North American growers ranked common scab as the fourth most important disease of potato (Loria *et al.*, 1997). In Tasmania it is regarded as the most important disease of potatoes (Pung, 1997).

Potatoes are a major vegetable crop produced in Tasmania and are predominantly grown on the fertile, basalt-derived krasnozem soils of the state's north-west. Prior to the adoption in the mid 1980s of Russet Burbank as the preferred processing cultivar, common scab was not considered a significant problem by the Tasmanian potato industry (Anon., 1954; Chapman, 1978). Previously Kennebec had been the main cultivar used for processing (Fountain, 1968; Chapman, 1977). Irrigation practices used with Kennebec were sufficient to keep common scab under control (Chapman, 1978). Kennebec is susceptible to powdery scab, caused by the fungus *Spongospora subterranea* (Wallr.) Lagerh. which is also present in Tasmania.

With the introduction of Russet Burbank there has been a significant increase in incidence of common scab, to the extent that it has become the disease of most concern to the potato industry. It may appear curious that this cultivar is susceptible to common scab in Tasmania as it is regarded elsewhere as being moderately scab resistant (Powelson *et al.*, 1993).

As is the case in other parts of the world, the factors affecting the incidence of common scab in Tasmania are not fully understood. Research conducted in recent years has attempted to elucidate some of

the local factors affecting disease incidence. Growers would like to know if a soil is going to be scab-promoting prior to sowing so that they can either avoid sowing affected areas or be ready to use other measures to control the disease. Consequently, potato cropping soils of North-West Tasmania were surveyed in 1994 by the Tasmanian Department of Primary Industry, Water and Environment (DPIWE) for soil factors associated with common scab (Wilson, 1996). This survey found no single clearly discernible scab conducive factor amongst those measured although a weak negative correlation between soil electrical conductivity and scab severity was noted. A similar common scab survey was again conducted in 1995. Soil and tuber samples collected in the 1995 survey were evaluated in the current project.

It has not been known for certain what pathogen species are causing common scab disease in Tasmania. Although it had been assumed that *S. scabies* is the pathogen responsible, evidence from elsewhere indicated that this may not necessarily be the case. In a study by Ransom and Gilliam (1991) scab promoting pathogens were isolated but their identity was not conclusively determined.

Green manure crops are increasingly being used in association with potato cropping. These are grown over winter as a cover crop and incorporated into the soil prior to sowing the potato crop. There are anecdotal reports of green manures being associated with either increases or decreases in scab severity. However, there had been no previous controlled study of the effects of green manures on severity of common scab in the Tasmanian potato cropping region.

Following this introduction and a chapter on introductory literature (Chapter 2), the experimental work constituting this thesis is presented in chapters 3 to 6. Chapter 3 lists general methods and details of pilot studies on soil enzyme assays. Chapter 4 covers a study on *Streptomyces* strains isolated from scab lesions of potatoes from the Tasmanian potato-growing region. Aims were to identify the species present and to identify pathogens and potential antagonists amongst the strains. Chapter 5 covers a study of a selection of soils from the potato growing region, the aim of which was to determine whether any of 12 soil chemical properties were associated with increased scab incidence. This chapter includes a re-evaluation of data from the 1994 DPIWE scab survey. Chapter 6 covers a study of the effects of green manure crops on common scab incidence and on the soil in general. Aims of the green

manures work were to determine whether green manures could affect scab severity in Tasmanian potato-cropping soils and to determine the effect of incorporation of a streptomycete antagonist with the green manures. The relevant research aims are further discussed in the introductions to Chapters 4 to 6. A summary of findings (Chapter 7) draws together issues relating to these three experimental sections.

Any reference to scab in this thesis, unless otherwise noted, refers to common scab.

Part of the results of this thesis has been published:

M.J. Lacey and C.R. Wilson (1997) Effects of green manures and a microbial antagonist on incidence of potato common scab in a Tasmanian soil. American Phytopathological Society Annual Meeting, Rochester, New York. *Phytopathology* 87:S55 (abstract).

2 Literature

2.1 Identity of scab promoting organisms.

Streptomyces scabies is the species historically associated with common scab of potato and is the major causal agent for this disease. It was first identified in 1890 by Thaxter who named it *Oospora scabies* (Thaxter, 1891, 1892; Lambert and Loria, 1989). It was characterised as producing grey spores in spiral chains and with the production of melanin in culture, however no type culture was maintained. Later this species was renamed *Actinomyces scabies* (Güssow, 1914) and subsequently *Streptomyces scabies* (Waksman and Henrici, 1948). Waksman, in 1961, redescribed the species (Waksman, 1961) and erroneously designated a non-typical stain (IMRU 3018) as the neotype strain. This strain differed from Thaxter's original description in lacking spiral spore chains and melanin production. As a result of this error the taxonomic designation of organisms causing common scab remained confused for many years. In the International *Streptomyces* Project (ISP) of the 1960s strain IMRU 3018 represented *S. scabies* . This strain represented *S. scabies* in all of the keys that arose from ISP (Küster, 1972; Nonomura, 1974; Szabó *et al.*, 1975).

Consequently the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974) listed *S. scabies* as invalid, because the type strain did not exist and strains ascribed to the species were taxonomically diverse. Many of these strains have subsequently been re-designated to other species.

Recognising the need to correct the description of *S. scabies,* Elesaway and Szabó (1979) revised Thaxter's original description, expanding it to include smooth spores and use of all ISP sugars, and offered a neotype strain (ATCC 33282). However *S. scabies* was not included on the list of approved bacterial names (Skerman *et al.*, 1980) which was published shortly after, resulting in continued uncertainty. The species was again revised by Lambert and Loria (1989a) who demonstrated that the majority of pathogenic strains they isolated from potatoes conformed to the description proposed by Elesaway and Szabó. A strain offered by Lambert and Loria (ATCC 49173) is now accepted

as the neotype. Strains of *S. scabies* evaluated by Lambert and Loria did not grow in culture at pH 5.0 or below (Lambert and Loria, 1989a)

S. scabies is the most widespread of the scab producing streptomycete pathogens, being found wherever potatoes are grown worldwide. Disease symptoms on potato range between superficial russetting, raised corky (erumpent) and deep pitted lesions. Similar lesions are caused on other root crops such as turnip, carrot, parsnip radish and beet (Hooker, 1981; Powelson *et al.*, 1993)). *S. scabies* has also been reported to cause pod wart of peanuts (Kritzman *et al.*, 1996). Common scab is generally not observed where soil pH is below 5.2 (Waksman, 1921).

In addition to *S. scabies,* other *Streptomyces* species have been shown to or reported to cause common scab or other scab-like diseases of potato. Three of these (*S. acidiscabies*, *S. caviscabies* and *S. turgidiscabies*) have recently been described (Lambert and Loria, 1989b; Goyer *et al.,* 1996a; Miyajima *et al.,* 1998).

Acid scab, first reported in Maine in 1953 (Bonde and McIntyre, 1968) and restricted to north-eastern parts of the United States and eastern Canada is caused by *S. acidiscabies* (Manzer *et al.*, 1977; Lambert and Loria, 1989b; Faucher *et al.*, 1992). Acid scab can cause disease in soils as low as pH 4.5 but with symptoms otherwise indistinguishable from common scab. *S. acidiscabies* can produce scab symptoms on other root crops (Loria *et al.*, 1997). The pathogen is morphologically and physiologically distinct from *S. scabies*. In culture *S. acidiscabies* produces white to pink spores in flexuous chains and a pH sensitive red to yellow diffusible pigment but not melanin. Strains of *S. acidiscabies* are also unable to utilise raffinose as a sole carbon source and can grow at pH 4.0. This pathogen does not appear to survive very well in soil and is spread by infected seed (Manzer *et al.*, 1977). It is believed that discontinuation of arsenic seed treatments in the early 1950s with substitution of the less toxic dithiocarbamate fungicides enabled the spread of this pathogen (Lambert and Loria, 1989b; Loria *et al.*, 1997).

Streptomyces caviscabies is a recently described species found to be associated with deep pitted scab lesions of potatoes grown on irrigated soil in Québec (Faucher *et al.,* 1992; Faucher *et al.,* 1995; Goyer *et al.,* 1996a). Isolated strains also caused pitted lesions in a

pathogenicity test (Faucher *et al.*, 1992). This species has been characterised (Faucher *et al.*, 1992; Faucher *et al.*, 1995) as having golden coloured mycelia and produced white spores in flexuous chains. Of the ISP sugars, only raffinose was used as a sole carbon source. Additionally, strains do not produce melanin or other diffusible pigments or grow at pH 4.5.

Streptomyces turgidiscabies causes potato scab in the Hokkaido region of Japan (Miyajima *et al.*, 1998). Lesions have been described as erumpent. In culture, *S. turgidiscabies* has a grey spore mass colour, with spores being produced in flexuous chains. No melanin or other diffusible pigments are produced and strains do not grow at pH 4.0. Strains used all of the ISP sugars. *S. turgidiscabies* infects a wide range root crops, producing symptoms similar to those of *S. scabies*. Strains of *S. turgidiscabies* have also been recently found in Finland (Kreuze *et al.*, 1999).

Other *Streptomyces* species have been associated with scab-like lesions of potato but are generally reported as being less virulent than *S. scabies*. These species include *S. griseus* (Corbaz, 1964; Hütter, 1967; Gordon and Horan, 1968), *S. aureofaciens* (Corbaz, 1964), *S. flaveolus* (Millard and Burr, 1926; Corbaz, 1964), *S. olivaceus* (Hütter, 1967). Archuleta and Easton (1981) reported that deep pitted scab in Washington state was caused not by *S. scabies* but by four other *Streptomyces* species (*S. diastatochromogenes, S. atroolivaceus, S. lydicus* and *S. resistomycificus*). However their findings have not been confirmed and their isolates have not been available for independent study (Faucher *et al.*,1995).

2.2 Phylogeny of pathogenic species

A number of phylogenetic studies of pathogenic species have been conducted, using various criteria including: morphological and physiological characteristics (Lambert and Loria 1989a; Lambert and Loria 1989b; Tashiro *et al.*, 1990; Goyer *et al.*, 1996b), DNA-DNA hybridisation (Tashiro *et al.*, 1990; Healy and Lambert, 1991; Goyer *et al.*, 1996b), cellular fatty acids (Paradis *et al.*, 1994; Ndowora *et al.*, 1996), 16S rRNA (Takeuchi *et al.*, 1996; Kreuze *et al.*, 1999). As a result of these studies it was shown that *S. scabies, S. acidiscabies, S. turgidiscabies* and *S. caviscabies* are separate species, being phenotypically distinct and sharing a low level of DNA homology.

S. turgidiscabies and *S. acidiscabies* are both genetically uniform (Miyajima *et al.*, 1998; Lambert and Loria 1989b). Lambert and Loria (1989b) speculated that the uniformity of strains of *S. acidiscabies* indicated they were from a common source. *S. scabies* strains, by comparison, have been found to be genetically diverse, indicating that pathogenicity arose in this species at a much earlier time. Healy and Lambert (1991) observed that the DNA diversity amongst *S. scabies* was greater than expected within a species. Ndowora *et al.* (1996) found that *S. scabies* strains isolated in the United States could be divided into two groups based on cellular fatty acid analysis. Paradis *et al.* (1994) found that eastern Canadian strains of *S. scabies* could be clustered into two groups, based on fatty acid composition. DNA-DNA hybridisation studies also indicated that these strains consisted of two distinct but genetically diverse groups, however there was no correlation between the DNA and fatty acid data. Pathogenic strains of *S. scabies* could not be distinguished from non-pathogens based on DNA or fatty acid data.

Takeushi *et al.* (1996) suggested that the lack of relationship between *S. scabies, S. acidiscabies* and *S. turgidiscabies* indicated that pathogenicity had arisen independently in each species. The possibility that pathogenic strains may have arisen by transfer of pathogenicity genes between unrelated strains has been suggested by a number of researchers including Tashiro *et al.* (1990), Healy and Lambert (1991), Doering-Saad *et al.* (1992) and Healy *et al.* (1999).

2.3 Aetiology

Infection of tubers occurs via lenticels during tuber initiation and early tuber enlargement (Adams and Lapwood, 1978). Symptoms first appear as necrosis around the infection site (Fellows, 1926) and lesions subsequently develop a corky appearance due to suberisation of surrounding tuber tissue. Individual lesions are normally circular but when many are present on a tuber they may coalesce to form irregular shaped scabbed areas. It also infects fibrous roots of potatoes and of other plant species (Hooker, 1949) causing local necrosis or reduced root growth.

Soil moisture during tuber formation is known to have a dramatic effect on scab development. Scab formation can usually be inhibited by maintaining soil moisture at or near field capacity for a period of 2 to 6 weeks following tuber initiation (Adams and Lapwood,

1978). This may be achieved by irrigation where soil moisture is inadequate. It is possible that reduced infection rates in wetter soils could be due to higher populations of competing bacteria in those soils (Lewis, 1970; Adams and Lapwood, 1978). Soils that are prone to dry out readily, such as sandy soils, tend to be more conducive to scab.

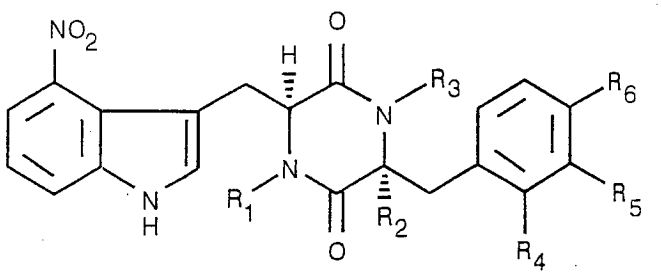
Until recently, little was known about the mechanism of pathogenicity in *S. scabies* and other species causing common scab. Involvement of a diffusible toxin or enzyme as a likely cause of symptom development was first suggested by Fellows (1926), this theory being supported by other studies (Jones, 1931; Shoemaker, 1952; Sakai *et al.*, 1984). Shoemaker (1952) found that scab-like lesions could be produced on the surface of tubers by placement of agar blocks taken from the vicinity of *S. scabies* cultures. Extracellular enzymes have been considered as a potential pathogenicity factor. An extracellular esterase, produced by *S. scabies* and capable of degrading suberin, was considered as a potential pathogenicity factor by McQueen and Schottel (1987). However not all pathogenic strains produced this enzyme and the importance of this and other esterase enzymes as a determining factor in pathogenicity in pathogenicity was not conclusively shown (Schottel, 1995).

Recent attention has focussed on a group of phytotoxic compounds, (named thaxtomins after Roland Thaxter who first described *S. scabies*) as a probable determining factor in pathogenicity of *S. scabies, S. acidiscabies* and *S. turgidiscabies*. These compounds which could replicate the early symptoms of common scab on immature tubers , first isolated and identified by King *et al.* (1989), are structurally defined as 4-nitroindol-3-yl containing 2,5 dioxopiperazines. (Figure 2.1)

Thaxtomin A (compound 1, Figure 2.1) is the predominant phytotoxin produced by *S. scabies, S. acidiscabies* and *S. turgidiscabies* (King and Lawrence, 1996; Bukhalid *et al.*, 1998). Ten other structurally related compounds are known to be produced in minor quantities (King and Lawrence, 1996). Thaxtomin C (compound 3, Figure 2.1) is the predominant thaxtomin produced by *S. ipomoea*, the species which causes soil rot of sweet potato (King *et al.*, 1994). The presence of the nitro group in the 4 position on the indole ring and presence of the phenyl side chain are required for phytotoxicity (King *et al.*, 1992). All known thaxtomins are yellow in colour (King and Lawrence, 1996).

The proposal that thaxtomins are responsible for pathogenicity has been supported by studies which show correlations between scab symptoms on potato minitubers and thaxtomin production by *S. scabies* (King *et al.*, 1991; Loria *et al.*, 1995) and *S. ipomoea* strains (King *et al.*, 1994).

A biosynthetic pathway for thaxtomin synthesis in *S. scabies* has been proposed (Babcock *et al.*, 1993; King and Lawrence, 1996).



Compound	R_1	R_2	R_3	R_4	R_5	R_6
1	Me	OH	Me	H	OH	H
2	Me	OH	Me	OH	H	H
3	Me	H	H	H	H	H
4	Me	OH	Me	H	H	H
5	Me	H	Me	H	H	H
6	Me	OH	H	H	H	H
7	Me	OH	Me	H	H	OH
8	Me	OH	Me	H	OH	OH
9	Me	OH	H	H	OH	H
10	H	OH	Me	H	OH	H
11	H	H	H	H	H	H

Figure 2.1 Structure of thaxtomins produced by plant pathogenic *Streptomyces*. (Figure reproduced from Loria *et al.*, 1997)

Leiner *et al.* (1996) found that inoculation with *S. scabies* significantly inhibited growth of seedlings of 11 out of 14 plant species tested. Symptoms included reduction in shoot and root growth, tissue thickening and necrosis. Symptoms could be reproduced by supernatants from the *S. scabies* cultures and indistinguishable symptoms could be elicited by thaxtomin A. The effect of thaxtomin A on seedlings varied with concentration. At lower concentrations shoot and root stunting and necrosis were observed. This ranged at higher concentrations to greatly

reduced growth, tissue necrosis and seedling death. Seedlings did not develop root hairs if they were not already present before thaxtomin was applied. Thaxtomin A was observed to promote cell hypertrophy, prompting speculation that its mode of action was on the cytoskeleton.

2.4 The genetic basis of pathogenicity

Bukhalid and Loria (1997) have cloned a gene (*nec1*) from *S. scabies* which allowed an otherwise non pathogenic *S. lividans* strain to cause necrosis of and colonise potato slices and to cause scab-like lesions on immature potato tubers. The *S. lividans* strain expressing *nec1* did not produce thaxtomin A but did produce an unidentified phytotoxic substance. Uncharacteristic of *Streptomyces* species, *nec1* has a low G+C (guanine +cytosine) content, indicating that this gene had been acquired via horizontal transfer from another genus. A strong correlation was found between presence of *nec1* and thaxtomin production by strains of *S. scabies, S. acidiscabies* and *S. turgidiscabies* (Bukhalid *et al.,* 1998). In close association with *nec1* in pathogenic species (Figure 2.2) are two genetic elements designated ORFtnp and IS1629 (Healy *et al.*, 1999). ORFtnp is a IS256 transposase homolog and is apparently non-functional due to a frameshift mutation. IS1629 is a insertion sequence element with homology with other insertion sequences found in a number of bacteria pathogenic to animals and encodes a transposase (Healy *et al.*, 1999). IS1629 is present in some *S. scabies* strains (Type II in Figure 2.2) as well as *S. acidiscabies* and *S. turgidiscabies* (Bukhalid *et al.,* 1998). An additional copy of IS1629 is present between ORFtnp and *nec1* in *S. acidiscabies* strains. The genetic organisation of the *nec1*-ORFtnp-IS29 region is highly conserved and strongly suggested the unidirectional transfer of a pathogenicity island containing these genes from *S. scabies* to *S. turgidiscabies* and *S. acidiscabies.*

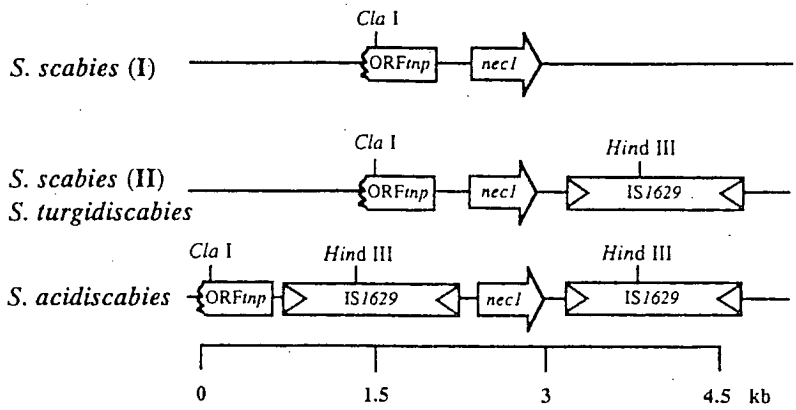


Figure 2.2 Genetic organisation of ORFtnp-*necI*-IS1629 region in *S. scabies* (type I and II) *S. acidiscabies* and *S. turgidiscabies strains.* (Figure reproduced from Healy *et al.*, 1999).

2.5 Pathogenicity testing

A range of methods have been developed for assessing pathogenicity of *Streptomyces* strains suspected of causing common scab. The traditional method involves attempted replication of scab symptoms on tubers produced on potato plants grown from seed pieces in a medium infested with the test strain (Labruyere, 1971; Loria and Davis, 1988). In a modification of this method, potato stem cuttings can be used as a source of minitubers, which are produced at axillary buds (Loria and Kempter, 1986). Testing for pathogenicity using whole potato plants can, however, be laborious and time consuming.

A potato slice assay (also called potato disk assay) has been proposed by Loria *et al.*, (1995) as a convenient means to identify pathogenic *Streptomyces* strains and to detect thaxtomins in growth media. It had been observed that thaxtomins could cause necrosis of potato tuber slices. Similarly cultures of pathogenic strains were observed to grow on and cause necrosis of tuber slices.

2.6 Scab suppressive soils and biological control

There are a number of examples of the development of scab suppressiveness of soils following many years of potato monoculture

(Weinhold *et al.*, 1964; Menzies, 1959). One such site at Grand Rapids, Minnesota had been established in 1942 for evaluating scab susceptibility of potato breeding lines. This site was abandoned after 30 years as it was no longer scab conducive, even to susceptible cultivars (Lorang *et al.*, 1989). Menzies (1959) proposed that a biological factor was responsible for scab suppressiveness of a soil in Washington. He demonstrated that the suppressiveness could be transferred to conducive soil by mixing-in 1% suppressive soil and 1% alfalfa (lucerne). Antibiotic producing *Streptomyces* strains antagonistic to *S. scabies* have been isolated from the Grand Rapids site (Lorang *et al.*, 1989; Liu *et al.*, 1996) and have been the subject of much research at the University of Minnesota into the nature of scab suppressiveness. One aspect has been the investigation of the use of scab antagonists as bio-control agents (Liu *et al.*, 1995 and 1996). Liu *et al.*, (1995) was able to demonstrate biological control of common scab by two suppressive strains (*S. diastatochromogenes* strain PonSSII and *S. scabies* strain PonR) in a 4-year field-pot experiment. The strains had been grown in a vermiculite-oatmeal medium and added to scab conducive soil at 1, 5 and 10 % (vol/vol). Disease control associated with PonSSII added at 1% increased each year and after the first year neither strain added at 1% showed significantly different control to inoculum added at 5 or 10%.

An alternative approach to biological control has been demonstrated in Japan by Hayashida *et al.* (1989). An antibiotic biofertilizer produced by growing *S. albidoflavus* strain CH-33 on swine faeces was found to inhibit *S. scabies* and scab development in pot and field trials.

The promising results of biocontrol experiments demonstrate their potential application for disease control. Although control may be demonstrated in pot experiments or small-scale field trials there are, however, considerable difficulties associated with the reliable and economical production and application of bio-control agents for commercial field scale control of soil borne diseases (Fravel, 1999). An important difficulty is the large (and possibly uneconomic) volume of inoculum that would need to be produced for effective field scale application. Potential effects on the existing microbiota of the target soil and on the health of people producing or applying biocontrol organisms also need to be taken into consideration.

2.7 Plant nutrients and scab

The influence of plant nutrients on incidence of common scab of potato has been reviewed by Keinath and Loria (1989). To a large extent, experimentally determined effects of nutrients on scab can be explained as being a result of associated changes in soil pH, or of the effect that the soil's pH has on those nutrients. Thus control of scab by addition of sulphur (Wheeler and Adams, 1897) can be explained by the associated reduction in soil pH which can be reversed by application of lime ($CaCO_3$), (Larson *et al.*, 1938). Generally the effect of calcium addition in increasing scab can be correlated with an increase in pH , while beneficial effects of nitrogen addition on scab appear to be mainly due to soil acidification (Keinath and Loria, 1989). Depending on their Ca content, phosphate fertilisers may increases soil pH and thus scab. Other elements that have been investigated include, iron, zinc, copper, boron and manganese. Mn (Halliwell, 1978), B, Cu and Fe are involved in lignin synthesis in plants (Marschner, 1986). Copper application can reduce scab incidence but is phytotoxic and therefore not used for scab control (Keinath and Loria, 1989). Studies of B, Fe, and Zn have generally been inconclusive or have shown no effect on scab severity. In some cases manganese application has been associated with scab reduction (eg. McGregor and Wilson, 1966) but in other cases no effect has been found (Keinath and Loria, 1989).

A number of researchers have suggested that measures of calcium are more indicative of scab than pH (Horsfall *et al.*,1954; Goto, 1985; Davis *et al.*, 1974, Davis *et al.*, 1976). However these findings have been refuted by Lambert and Manzer (1991). Horsfall *et al.* (1954) found that scab severity was positively related with calcium content of tubers. They proposed a causal relationship of calcium in scab development but were unable to determine a mechanism by which this could occur. Their data was later analysed by Lambert and Manzer (1991) who concluded that it showed no relationship between calcium and scab. Lambert and Manzer (1991) backed up their assertion with their own finding that high Ca in tuber periderm tissue was an effect of scab rather than a cause. Similarly an assertion by Goto (1985) that calcium was a better indicator, than pH, of scab severity in one of two soils has been dispelled by Lambert and Manzer (1991) who concluded the converse.

Schroeder and Albrecht (1942) observed that application to soil of either potassium or calcium could increase scab and that potassium had a greater scabbing effect than calcium. Gries *et al.* (1944) proposed that the soil Ca:K ratio was an important factor in scab conduciveness. However Doyle and MacLean (1960) found no relationship, independent of soil pH, between scab and Ca:K ratios. Other studies have found no relationship between potassium and scab (Gusenleitner, 1974; Reichard and Wenzl, 1976; Wenzl and Reichard, 1974).

2.8 Green manuring and common scab

There have been several studies looking at the effects of green manure crops on common scab. Findings of these studies show no consistent trend, with some researchers reporting reductions in disease incidence (Weinhold *et al.*, 1964; Rouatt and Atkinson 1950; White, 1928; Millard, 1923) while others have found no effect (Sanford, 1926; Goss, 1937; Dippenaar, 1933; Divis and Kristufek, 1998) or even increased scab severity associated with green manures (Weinhold and Bowman, 1968; KenKnight, 1941). Differences in experimental outcomes could reflect variations in any of many factors such as type of green manure crop, the amount applied, timing and method of application, soil type, site specific factors, climate and experimental procedure. In addition it appears that any effects of added green manures may not necessarily be restricted to the following crop but may exerted over a longer period or at a later time (eg. Weinhold *et al.*, 1964). Longer term field experiments such as that of Weinhold *et al.*, (1964) may be a more reliable indicator of the influence of green manures than single season field experiments or glasshouse experiments.

Weinhold *et al.* (1964) looked at green manures and crop rotations in a 13 year field study. In one part of their study they compared the effect of six different crop rotations on scab severity. Continuous cropping with potatoes over the period was associated with an increase in scab severity with a maximum severity being reached at eight years. Cropping in alternate years with barley, cotton or sugar beet was also associated with increases in scab severity although to a lesser extent than continuous potato cropping. They speculated that the level of scab was primarily dependent on the number of preceding potato crops. A three year potato-sugar beet-cotton rotation appeared to promote a more rapid increase in scab severity than the two-year rotations or

continuous potato cropping. In another part of their study, potato crops were grown each year with a cover crop in between. These cover crops were incorporated into the soil as a green manure. It was observed that where barley was used as the green manure crop the scab incidence in subsequent years increased to a greater level than for the control (without a green manure). In contrast, crops with a soybean green manure showed no increase in scab over the period of the experiment. They also observed that although soybean green manure crop prevented an increase in scab severity it was not effective in reducing scab if grown where scab incidence was already high.

A number of researchers (Millard and Taylor, 1927; Rouatt and Atkinson, 1950; Weinhold and Bowman, 1968) have suggested that observed beneficial effects on scab severity with green manures could be due to stimulation of antagonistic micro-organisms. Weinhold and Bowman (1968) found that bacteria identified as *Bacillus subtilis* commonly isolated from the trial plots were antagonistic to *S. scabies* and increased in numbers during decomposition of green manures in the soil. Rouatt and Atkinson (1950) studied the effects of green manures on potatoes grown in sandy soil in a glasshouse experiment. Incorporation of soybean was reported to be associated with reduced scab while pea or rye had no apparent effect. They speculated that the reduced scab incidence was associated with a change in soil microbiota. However the observed reduction in scab and changed soil microbiota with soybean might also be accounted for by a reduction in soil pH to 5.0 compared to $\geq$ 6.4 for the other treatments. In a series of pot experiments Millard and Taylor (1927) added grass clippings and various amounts of *S. scabies* and *Actinomyces* (syn. *Streptomyces*) *praecox* inoculum to sterilised soil. In the absence of *S. praecox*, grass clippings had no effect in scab severity but where *S. praecox* had been added scab severity was reduced.

3 General Methods and Pilot Studies

3.1 Introduction - soil assays

A number of glasshouse and field experiments involved amendment of soil with organic matter and/or microbial inoculum. In addition to possible effects that they may have on potato scab, it was believed that these amendments also may have other underlying effects on soil properties which might be correlated with scab severity. For this reason assays were also conducted, in the relevant experiments, of a number of soil properties thought likely to have some bearing on scab severity. These assays included soil pH, electrical conductivity and two measures of soil enzymatic activity. In some experiments plate counts of microbiota (particularly actinomycetes) were determined.

Soil pH and electrical conductivity were monitored as they have been shown previously to have some bearing on scab incidence. The relationship between soil pH and scab severity is well documented (Waksman, 1921). Electrical conductivity was monitored as it appeared to be correlated with scab severity in the 1994 DPIWE potato scab study (Wilson, 1996). Electrical conductivity is used to estimate the concentration of soluble salts in soil. Assays of soil enzyme activity were used to give a measure of soil microbial activity.

Two enzyme assays were chosen, these being cellulase and fluorescein diacetate (3',6' –diacetylfluorescein; FDA) hydrolysis. Cellulase was considered a useful enzyme to measure the rate of decomposition of cellulosic vegetable material added to soil in green manures experiments.

FDA can be hydrolysed by many different enzymes, including esterases, lipases and proteases. The reaction product is fluorescein, which can be readily assayed by spectrophotometric or fluorometric methods. The ability to hydrolyse FDA is widespread amongst soil inhabiting microbiota and as this reaction is not specific to a particular enzyme it has been proposed as a good general measure of microbial activity in soil (Schnürer and Rosswall, 1982; Dick, 1994).

A wide range of enzymes can be detected in soil (Ladd, 1978) and assays of their activities can give a good indication of the relative microbial activity. Although there is indirect evidence that they are predominantly of microbial origin (Skujins, 1978) soil enzymes may also originate from plant or animal sources.

Numerous assays have been devised for assessing activities of the soil enzymes. Depending on the enzyme being assayed, such activities may either represent a measure of the general biological activity of the soil or a measure of specific processes in nutrient cycling. In general soil enzyme activities do not correlate with microbial numbers present in those soils (Skujins, 1978).

Functional enzymes in the soil can be found: in living and dead cells; and exocellularly where they may be present in the soil solution, bound to soil particles or as a component of particulate cell debris. Assays generally measure total accumulated enzymes in a soil sample.

3.2 Soil pH and electrical conductivity

Soil pH and electrical conductivity were measured using Australian standard methods (Rayment and Higginson, 1992). Air dried soil samples (20.0 g) were suspended in distilled water (1:5 soil/water) in plastic containers. Containers were shaken for 60 minutes in end-over-end shaker. The pH was measured with a glass electrode, with agitation during measurement. Samples were then allowed to settle for 30 minutes before measurement with a conductivity meter. Calibration of the meter was with a 0.010 M KCl reference solution.

3.3 Assays of soil enzyme activity

3.3.1 Fluorescein diacetate (FDA) hydrolysis

FDA hydrolysis measurement was based on the method used by Song and Bartha (1990) with a number of modifications.

FDA was dissolved in acetone (1 $mgml^{-1}$) and stored at –20°C.

Each soil sample was sieved (2 mm mesh) and 3.0 g (moist weight) weighed-out into a 125 ml screw-top bottle. To this, 50 ml of 60 mM phosphate buffer (pH 7.6; Appendix 1.2.1) was added before

bottles were placed into 25°C water bath (Ratek Instruments) and allowed to equilibrate for 15 minutes. Next, 1.0 ml of the FDA stock solution was added to samples, which were then incubated for 60 minutes in the water bath, with gentle shaking. Subsequently, 5.0 ml of each sample was transferred to a screw-cap diluent tube (to which 5.0 ml of acetone has been previously added to stop FDA hydrolysis). Tubes were centrifuged at 5000 rpm for five minutes. Absorbance of supernatant at 490 nm (A_{490}) was measure on a spectrophotometer (Bausch & Lomb, Spectronic 20) using 1:1 buffer/acetone solution as a blank. FDA hydrolysis activity was calculated on a soil dry-weight basis.

3.3.1.1 FDA assay standardisation and calibration

Inclusion of a soil standard allowed cross comparison between assays. The soil standard was prepared from 1 kg of krasnozem soil which was air dried then sieved to exclude particles greater than 0.5 mm. This soil was then ground to a finer consistency with a mortar and pestle. The prepared soil was then stored in a sealed container at –20°C.

To confirm the expected effect of incubation time and sample size on absorbance readings (Schnürer and Rosswall, 1982), a pilot assay was also conducted with 1, 2 and 4 g of the soil standard, incubated for 0, 30, 60 and 120 minutes. Although there tended to be a nonlinear relationship between sample weight and associated absorbance readings (Figure 3.1) the choice of a sample weight of 3.0 g and incubation time of 60 minutes allowed this effect to be minimised in subsequent experiments. With these chosen conditions, sufficient variability was obtained with minimal skewing of higher absorbance figures.

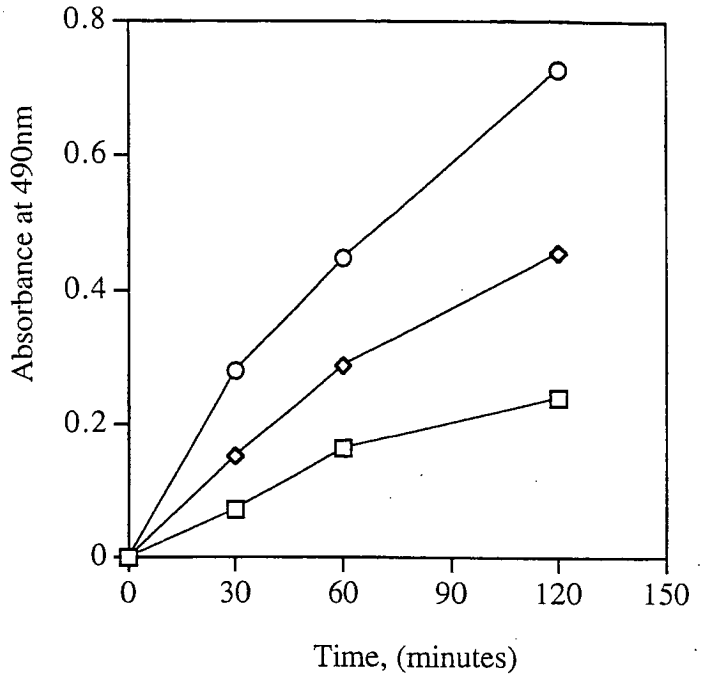


Figure 3.1 Hydrolysis of FDA with time by: 1 g, (□); 2 g, (◇); or 4 g, (○) of soil standard incubated in 50 ml of 60 mM phosphate buffer at pH 7.6.

3.3.2 Cellulase activity

Cellulase activity was measured according to the method of Braid and Line, (1980)

Soil samples were sieved (2 mm mesh) prior to analysis. Samples of moist soil (2.0 g) were incubated in 10.0ml of 0.4% carboxy-methyl cellulose (NaCMC) solution buffered at pH 5.5 (Appendix 1.2.2) in a water bath for 60 minutes at 45°C with gentle shaking. Cellulase activity was then halted by placing samples on ice (O°C), thus allowing them to be tested in batches.

Viscosity of 1.0 ml sample aliquots was determined using a Wells-Brookfield microviscometer, running at 30 rpm for 2.0 minutes at 25°C.

Percentage reduction in viscosity was calculated using the following formula:

$$\text{Reduction}\% = \left(\frac{c-s}{c-w}\right)\cdot 100$$

Where c= viscosity of control,

s= viscosity of assayed sample

w= viscosity of water

A pilot assay (Table 3.1) confirmed the expected effect of incubation temperature on rate of reduction in viscosity (Braid and Line, 1980). In this assay 2.0 g soil samples were incubated for 60 minutes at 25, 33, 45 or 55°C. Reduction in viscosity was optimal at 45 and 55°C but negligible at 25°C.

Table 3.1 Influence of temperature on rate of reduction in viscosity of NaCMC

Temperature, (°C)	Viscosity reduction, (%)
25	0.6
33	12.2
45	27.5
55	29.3

3.4 Actinomycete counts

3.4.1 Method used in green manures pot trial 1

Soil samples taken at harvest were plated onto carboxymethylcellulose agar (CMCA; see below) and GAT agar (Appendix 1.1.2). One gram of air dried soil was suspended in 10 ml of 0.85% saline at 25°C for 30 minutes with gentle shaking. Serial dilutions were spread onto plates which were then incubated for seven days at 25°C

3.4.1.1 Carboxymethylcellulose agar, (CMCA)

A cellulose medium was devised for enumeration of cellulose degraders. This was based on Glycerol-asparagine medium (Appendix 1.1.1) with carboxymethylcellulose substituted for glycerol. Ingredients were as follows:

Ingredient	Amount
Agar	15 g
Carboxy-Methyl Cellulose, sodium salt (BDH)	10 g
L- Asparagine (Sigma)	1 g
K_2HPO_4	1 g
Trace Salts Solution (see Appendix 1.1.13)	1.0 ml
Distilled water	1000 ml

Medium preparation procedure was as for Glycerol Asparagine Agar (Appendix 1.1.1)

3.4.2 Method used to count soil acinomycetes in other experiments

Following a modification of the method used by Lawrence (1956) for isolating actinomycetes from tubers, 1.0 g of air dried soil suspended in 10 ml of sterile1:144 phenol/water solution was shaken on a vortex mixer for four minutes and left to stand for one minute before serial dilutions were spread onto plates of GAT agar. Plates were dried for ten minutes in a laminar flow cabinet then incubated at 25°C for seven days.

3.5 Vegetation assay methods

Dry weights were determined on representative subsamples of vegetation used as green manures at the time of application to soil. Samples were dried for 12 hours at 65°C.

Percentages of carbon and nitrogen were also measured using a Carlo Erba EA1108 elemental analyser for which air dried samples were ground to a fine consistency.

3.6 Preparation of potato seed for pot trials

Unless otherwise noted, eye cores were used as potato seed in pot trials described in this thesis. Potato tubers were washed to remove any adhering soil then selected for absence of scab or other surface blemishes. Using a 12 mm cork borer, 2 cm long cores encompassing eyes were taken from tubers. Eye cores were washed in a 2% sodium hypochlorite solution for 2 min followed at least three rinses with tap water. Cores were then placed on tissue paper and allowed to dry overnight before sowing the following day.

3.7 Glasshouse conditions for pot experiments

Glasshouse temperatures were generally maintained in the range 10 to 25°C. No fertilizer was added to pots unless otherwise noted. Details of pot size, irrigation regime and soil type are detailed in the methods for the individual experiments.

3.8 Statistics and computing

Statistical analysis was carried out using Systat 5.2.1. Fisher's Least-Significant-Difference test was used to make multiple comparisons between treatments where ANOVA indicated a difference at $p \leq 0.05$.

This thesis was written using Microsoft Word 98. Microsoft Excel 98 was used for spreadsheet functions. Other software used in thesis preparation included: Cricket Graph 1.3.2 and MS Excel 98 for graphs; BBedit Lite 4.1 for text and data conversions; Adobe PhotoShop 3.0.5 and Claris MacDraw Pro 1.5.3 for graphics. All computing was done on Macintosh computers.

4 Evaluation of Tasmanian Isolates

4.1 Introduction

In order to be able to control a pathogen it is useful to first understand its biology. An important first step is to be able to clearly identify that pathogen.

Given that Tasmania is geographically distant from other regions of the world for which scab pathogens have been characterised and also given that there is known variability elsewhere amongst pathogens capable of causing common scab symptoms it was considered possible that Tasmanian potato scab conducive strains may differ from those found elsewhere. Elsewhere wide genetic variability has been found amongst *S. scabies* strains (Healy and Lambert, 1991; Ndowora *et al.*, 1996; Paradis *et al.*, 1994). New pathogens have been identified in some parts of the world such as *S. acidiscabies* in parts of USA and Canada and *S. turgidiscabies* in the Hokkaido region of Japan. No detailed study has conclusively identified the species responsible for causing common scab throughout the potato growing region of Tasmania. As part of an earlier study Ransom and Gilliam (1991) isolated pathogenic strains but did not conclusively determine their identity.

As a starting point in the current study, streptomycete strains were isolated from scab lesions of potatoes collected from across the Tasmanian potato growing region. In addition to pathogens it was considered possible that some useful scab-antagonists might be found amongst the strains collected. No scab-suppressive soils have been identified in Tasmania. However, a collection of isolates from scab lesions could prove a useful alternative source of suppressive strains.

Goals of this part of the study were to: 1) identify the actinomycete species associated with common scab lesions of potatoes from commercial potato production areas of Tasmania; 2) evaluate the pathogenicity of those species, and; 3) investigate whether any of the isolated strains have potential as scab antagonists.

4.2 Methods and Materials

4.2.1 Source of strains

The majority of strains used in this study were sourced from tubers collected in the 1995 DPIWE scab survey with the exception of one strain from the 1994 survey (95/13/1A) and four strains (#23, #25, #27, and #32) from. an earlier collection by C. Gilliam. Strains, #27 and #32 have previously been shown to be pathogens and #23 scab suppressive (C. Wilson pers. comm.). Strain #32 is an Australian *S. scabies* type strain collected in Victoria. Twenty one strains from the 1995 scab survey were obtained from M. Stroman of DPIWE.

Remaining strains were isolated from scab survey tubers using the following procedure which was a modification of that of Lawrence (1956). Lesions aseptically excised from tubers were ground with a mortar and pestle and the resultant material was transferred to 10 ml of sterile 1:140 water/phenol solution. After ten minutes, 0.10 ml of this suspension was spread onto plates of Glycerol asparagine tyrosine agar (GAT; Appendix 1.1.2) which were then incubated for 14 days at 25°C. Representative colonies were subcultured on to yeast-malt extract agar (YME, Appendix 1.1.3).

Source data recorded for strains from scab survey tubers was source site, grower and lesion type. Lesion type was recorded as either erumpent (raised, corky lesion), russet (superficial, corky lesion) or pitted following the classification scheme of Loria (1991). No data was available on source cultivar as this had not been recorded at the time of collection. Nevertheless, Russet Burbank can be assumed to be the source cultivar in most cases as this was the predominant cultivar grown.

4.2.2 Maintainance and inoculation of strains

For general use, cultures were maintained on YME plates at 20°C. Inoculum used in experimental procedures was in the form of spores transferred with a wire loop from YME cultures, unless otherwise noted.

Strains were also stored as a spore suspension at –20°C in a 30% glycerol medium. Cultures were grown on YME for three weeks. Sterile storage medium (5 ml) consisting of 1/3 strength YME with 30%

glycerol was transferred to each plate and the surface scraped to loosen colonies. The resulting spore suspension was transferred to a storage vial (Nalgene).

4.2.3 Phenotypic characterisation of strains

Methods for characterisation of the strains generally followed that of Lambert and Loria (1989a). A detailed description of methods is as follows.

4.2.3.1 Spore colour

Colour of spore mass of mature colonies growing on YME and the colour of the reverse side of these plates was described simply.

4.2.3.2 Hygroscopic cultures

For some strains, plate cultures were observed to become hygroscopic resulting in the spore mass disintegrating to form a black mucoid liquid at about one month after inoculation. This was recorded for strains growing on YME.

4.2.3.3 Spore chain morphology

Petri plates with mature cultures were inverted and colonies examined directly under a microscope at low magnification (100x). Where spores were borne in chains these were described as being either flexuous (straight or wavy), loose spirals or tight spirals. Strains producing spores in sporangia were identified according to the descriptions of Goodfellow *et al.* (1984).

4.2.3.4 Pigmentation

Production of melanin on peptone yeast extract iron agar (PYI; Appendix 1.1.4) and on Tyrosine Agar (Appendix 1.5) was recorded after seven days with brown colouration around colonies being indicative of melanin production.

Presence of other diffusible pigments was evaluated on glycerol asparagine agar (Appendix 1.1.1) and, where present, the colour was recorded after 14 days.

4.2.3.5 Biochemical tests

For the following biochemical tests, spores taken from fresh YME cultures were spot inoculated onto plates of the test media. Each strain was assayed at least in duplicate. Plates were incubated at 25°C.

4.2.3.6 Utilisation of carbon sources

Utilisation of each of the nine International Streptomyces Project (ISP) sugars as sole carbon sources was assessed on a minimal medium (Appendix 1.1.6). Growth was assessed after 30 days, with glucose being used as a positive control.

4.2.3.7 Utilisation of nitrogen sources

Utilisation of L-hydroxyproline or L-methionine as sole nitrogen sources was assessed on a minimal medium (Appendix 1.1.7). Growth was assessed after 30 days.

4.2.3.8 Degradative activity

Degradation of xanthine, xylan and starch was evaluated on modified Bennett agar (Appendix 1.1.8) at 4.0, 4.0 and 10.0 g/litre respectively. Poygalacturonate degradation was evaluated using Hankin Pectin Medium (Appendix 1.1.9). Degradative activity was indicated by clearing of the media surrounding colonies after seven days. Starch plates were developed by flooding with Gram iodine solution. Pectin medium was developed by flooding plates for one hour with a warmed 1% (w/v) solution of hexadecyltrimethylammonium bromide.

Arbutin degradation was evaluated using the method of Kutzner (1976), as cited by Williams *et al.* (1983b). Each strain was tested on media with and without arbutin (Appendix 1.1.10). Observations were made after five and ten days. A positive result being the development of brown pigment near colonies in the medium containing arbutin but not in the corresponding arbutin-free control.

4.2.3.9 pH sensitivity

The minimum pH allowing growth was determined on media covering the pH range 3.5 to 6.5 in 0.5 unit increments (Appendix 1.1.11), according to the method of Lambert and Loria, (1989a).

4.2.3.10 Growth with NaCl

Growth inhibition by 5, 6 and 7% NaCl (w/v) was assessed using the medium of Kutzner (1981) (Appendix 1.1.12). Presence or absence of growth was recorded after ten days.

4.2.3.11 Growth inhibition by antibiotics, and other toxic substances

Inhibitory activities of antibiotics and other compounds was determined on modified Bennett agar. Substances chosen were those used by Lambert and Loria (1989a) ,with the exception of those where they had found no difference between strains, and included: tellurite (10 and 100 µg/ml) as potassium salt; thallium (10 and 100 µg/ml) as acetate; crystal violet (0.5 µg/ml); phenol (0.1% w/v); penicillin G (10 IU/ml); oleandomycin (25 and 100 µg/ml); and streptomycin sulphate (20 µg/ml). Growth was scored as presence or absence at 30 days.

4.2.4 Numerical taxonomy

To assist in grouping of strains, characteristics were compared according to Burr's strategy (incremental sum of squares with standard euclidean distance) using the program TAXON 1.0 (Ross and Shields, 1993). Characteristics which varied between strains were included in this analysis and were coded as: binary (presence/absence eg. melanin production) ; disordered multistate (where two or more possible states eg. spore colour); or numeric (eg. minimum growth pH).

4.2.5 Pathogenicity tests

Four assays were used to evaluate pathogenicity of strains. Initially all strains were screened using a potato disk assay. A selection of strains were re-examined using radish seedling and minituber assays. Pathogenicity of smaller selection of strains was also assessed on potato plants in a glasshouse trial.

4.2.5.1 Potato disk assay

The potato disk assay was based on the method of Loria *et al.* (1995). Immature tubers of cultivars Kennebec and Bismark were stored at 4°C for at least two months. Tubers were peeled and surface sterilised for two minutes in 1% sodium hypochlorite then rinsed in several changes of sterile water. Cores (1.2 cm diameter) aseptically taken from the centre of tubers were cut into 2.5 mm slices which were placed onto water agar in petri plates.

Strains were grown on oatmeal agar (Appendix 1.1.14) for six days at 25°C after which 3 mm^2 blocks of media with attached sporulating colonies were cut from plates and inverted onto potato disks. Each strain was tested on duplicate potato disks of at least one potato cultivar and most were tested in duplicate on both cultivars. Potato disks were incubated for up to six days at 25°C.

Potato disks were examined at 20 and 45 hours for signs of discolouration and other changes in appearance near where the agar block had been placed. The extent of growth and/or sporulation of the test strain on the potato disk was assessed at six days. Attachment of the agar block to the potato slice (an additional indication of growth of the test strain) and depth of necrosis were assessed by probing around and below the agar block with a pair of tweezers at day six.

4.2.5.2 Radish seedling assay

A radish seedling assay, based on that of Leiner *et al.* (1996), was used to cross-check pathogenicity for a selection of 26 strains. Strains selected included those considered most likely to be pathogens plus representatives from other groups.

4.2.5.2.1 Preparation of strains

Strains were grown for eight days in 100 ml oatmeal broth (OMB; Appendix 1.1.15) on a rotary shaker (110 rpm) at 15-30°C (with one broth culture per strain).

4.2.5.2.2 Preparation of seedlings

Seed of radish variety 'Fireball' was surface sterilized in 1% sodium hypochlorite for two minutes and then rinsed with three changes

sterile water. Seed was then pregerminated for one day on water agar. Germinated seedlings selected for uniformity in size were individually placed into sterilised glass tubes (28 mm diameter) containing 10 ml of 1.5% water agar.

4.2.5.2.3 Assay

Seedlings were inoculated with 0.125 ml of broth culture medium (previously centrifuged at 5000 rpm for five minutes and filtered [Whatman, grade 54] under vacuum). Controls received uninoculated OMB broth. Six seedlings were inoculated per strain tested.

Seedlings were then grown under lights, with a 12 hour photoperiod, at 24°C for six days after which they were assessed for necrosis and abnormalities in growth. Seedlings were then removed from tubes and shoot length was measured as the distance between the radicle and cotyledons.

4.2.5.3 Minituber assay

Pathogenicity of strains assessed by the radish seedling assay was reassessed using a potato minituber assay following a protocol of Fry (1997, pers. comm.) with modifications.

4.2.5.3.1 Minituber production

Potato plants of cultivar Kennebec were grown in 20 cm diameter pots in potting mix. Mature plants were selected after one month for tuber induction. To induce tuber formation, pots were transferred to a phytotron for 13 days with ten hour light period, with day time temperatures being 22°C and less than 18°C at night.

Leaf bud cuttings, each of which included leaf nodes with 1 cm of stem either side and the subtending leaf, were then prepared and placed in perforated plastic trays in vermiculite in a mist chamber for 10 -13 days.

At the end of this period, cuttings which had formed tubers (Figure. 4.1) in the range 0.5 to 1 cm diameter were selected for assay.

4.2.5.3.2 Inoculum preparation

The same oatmeal broth culture extracts used in the radish seedling assay were used in this assay. The filtered broth media had been stored at –20°C for up to one month and was thawed immediately prior to use. Aliquots of 20 ml were removed before the remainder was re-frozen.

4.2.5.3.3 Assay

The leaf was removed from each cutting and the minituber (with attached stem) rinsed in distilled water before being immersed in the broth medium for one minute. Each strain was tested with six or seven minitubers. Controls were immersed in uninoculated OMB or not immersed. Minitubers were placed under sterile conditions onto water agar in plastic trays which were then covered with aluminium foil and kept in the dark for four days. Minitubers were then assessed for scab severity.

Figure 4.1 Minituber formation on potato stem cuttings

4.2.5.4 Pot assay of pathogenicity of selected strains

This pot assay of pathogenicity was carried out according to the method of Labruyère (1971). Strains were grown in 250 ml flasks on vermiculite saturated with sucrose asparagine yeast extract (SAY; Appendix 1.1.16) solution. Flasks were incubated for three weeks at

30°C. Samples of inoculum were plated out onto YME to confirm purity and viability.

Strains were evaluated in sand in 16 cm diameter pots in duplicate (Figure 4.2). Polypropylene mesh was placed in the bottom of each pot to prevent medium from falling out. Pots were each filled to within 5 cm from the top with coarse sand. Vermiculite inoculum (40 cc) was mixed to a depth of 3 cm in each pot. Strain #32 was used as a positive control and inoculum-free vermiculite as a negative control. Two eye-cores of potato cv. Kennebec were sown per pot. Pots were spaced 40 cm apart to minimise cross contamination. Slow release fertilizer ('Osmocote'™, NPK= 10:4.8:15) and a trace elements solution was applied to pots at planting.

After three months, tubers were harvested and assessed for presence or absence of scab.

Figure 4.2 Pot assay to evaluate pathogenicity of strains on potato plants (cv Kennebec) grown in sand.

4.2.6 Analysis of extracts of culture media used in radish seedling and minituber assays for known phytotoxins

4.2.6.1 Chloroform extraction of media

Oatmeal broth medium, from which aliquots had previously been removed for the radish seedling and minituber assays, had been stored at -20°C for five months. Remaining medium (49 to 77 ml depending on sample) was extracted with chloroform using a modification of the method used by King *et al.* (1992). Each sample was extracted twice with equal volumes of chloroform (HPLC grade) in a separatory funnel. Chloroform extracts were then evaporated to dryness in Buchi Rotavapor under vacuum at 32°C. The resultant residue was dissolved in 1 ml methanol which was evaporated before storing at -20°C in glass vials. Samples were stored for a further 9 months before thin layer chromatography and mass spectrometric analysis.

4.2.6.2 Thin layer chromatography

Chloroform extracts were compared by thin layer chromatography (TLC) using a modification of the method of King *et al.*(1992).

Extracts, taken up in methanol, were spotted onto Merck silica gel 60 F_{254} plates which were run in 9:1 chloroform/methanol. Authentic thaxtomin A, originally provided by R.R. King, was included in plates as a reference marker. Bands on developed plates were observed under 254 and 366 nm UV light.

4.2.6.3 Mass spectrometry

A selection of extracts were examined by mass spectrometry (MS) to assist in identification of constituent compounds. For high resolution MS and MS/MS analysis a Kratos Concept ISQ High Resolution/Quadrupole Tandem Mass Spectrometer was used. Ionisation modes used with this machine were electron-impact ionisation (EI) and electrospray ionisation (ESI).

Some samples were also first fractionated with a Waters Alliance 2690 HPLC with constituents of the HPLC peaks examined directly by a Finnigan LCQ mass spectrometer or collected for later

analysis by the Kratos Concept ISQ. The Finnigan LCQ system was equipped with atmospheric pressure chemical ionisation (APCI) and EI ionisation sources. Ultraviolet (UV) spectra of individual HPLC peak could also be determined.

For simplicity of presentation, specific details of methods used in mass spectrometric analyses are included with the experimental results to which they relate.

4.2.7 Assessment of fresh OMB broth cultures for thaxtomin A

In experiments described earlier, selected strains had been grown in 100 ml of OMB and this broth had been used in radish seedling and minituber assays. Broth media had then been stored at -20°C for five months before chloroform extraction and then extracts further stored at -20°C for an additional nine months before assessment for the presence of thaxtomin A by TLC and mass spectrometry. In order to discount the effect of storage time, fresh OMB cultures were prepared and assessed immediately for thaxtomin A.

A selection of strains were each grown in a larger volume (500 ml) of oatmeal broth for 12 days at 20°C with shaking at 50 rpm. Media was then extracted with chloroform as described previously. Extracts were compared by TLC as described previously using, as a reference, an authentic thaxtomin A standard provided by G. Luckman.

4.2.8 Selection of antagonist strains for glasshouse studies

4.2.8.1 Co-plating assay

In order to select potential antagonists for use in glasshouse biocontrol studies a co-plating assay was used in which strains were screened against four strains believed at the time to be pathogens. Pathogens included three presumed pathogens as determined by the potato disk assay (strains 46/1A-1, 48/2 and 75/1-1, all subsequently identified as being strains of *S. violaceusniger*) and strain #27, also independently shown to be pathogenic (C. Wilson, pers. comm.).

Spread plates of pathogen strains on oatmeal agar were prepared using spores taken from mature oatmeal agar cultures. Spores of test strains (also from mature oatmeal agar cultures) were then spotted

around the edge of test plates at eight strains per plate in duplicate. Plates were incubated at 25 °C and were assessed at eight days. Width of inhibition zone, where present, was recorded. In addition the presence or absence of sporulation of the test strains and inhibition of lawn sporulation near the test spot were recorded.

Additional criteria taken into account when selecting antagonists included the minimum pH required for growth and whether the strain was regarded as non-pathogenic as determined by the potato slice assay.

4.3 Results

4.3.1 Strains

A total of 95 isolates were collected. Source site, potato grower and lesion type of 70 strains isolated from tubers are listed in Table 4.1. Each isolate from the1995 DPIWE scab survey was given an individual strain code with notation following the format: 'site'/'tuber'-'isolate number'. Multiple unique isolates were obtained from some tubers while not all tubers yielded isolates. An additional 25 strains obtained from M. Stroman of DPIWE are listed in Table 4.2. Source locations, where known, are also mapped in Figure 4.3.

Table 4.1 Strains isolated from tubers collected in the 1995 scab survey[1]

Strain	Source Site	Grower	Lesion Type
1/2-3	Waterhouse, Tas.	K. Lette	Russet
1/2-4	Waterhouse, Tas.	K. Lette	Russet
2/1-1	South Riana, Tas.	A. Clarke	Russet
2/1-3	South Riana, Tas.	A. Clarke	Russet
2/1-4	South Riana, Tas.	A. Clarke	Russet
2/1-5	South Riana, Tas.	A. Clarke	Russet
2/2-2	South Riana, Tas.	A. Clarke	Russet
2/2-3	South Riana, Tas.	A. Clarke	Russet
2/2-4	South Riana, Tas.	A. Clarke	Russet
2/2-5	South Riana, Tas.	A. Clarke	Russet
25/2	Deloraine, Tas.	R. Scott	Erumpent
41/2	Barrington, Tas.	C. Fitzmaurice	Erumpent
42/1-2	Moriarty, Tas.	D. Addison	Russet
42/2-1	Moriarty, Tas.	D. Addison	Russet
43/1-1	Railton, Tas.	S. Langton	Russet
43/1-2	Railton, Tas.	S. Langton	Russet
43/1-3	Railton, Tas.	S. Langton	Russet
44/1A-1	Penguin, Tas.	G. McKenna	Erumpent
46/1A-1	Moriarty, Tas.	N. Badcock	Erumpent
46/1B-1	Moriarty, Tas.	N. Badcock	Russet
46/2B	Moriarty, Tas.	N. Badcock	Erumpent
48/2	Barrington, Tas.	C. Fitzmaurice	Erumpent
49/1	Penguin, Tas.	G. McKenna	Russet
51/1	Riana, Tas.	L. Langham	Erumpent
51/2-1	Riana, Tas.	L. Langham	Russet
52/2	South Riana, Tas.	D. Carr	Pitted
53/2	West Pine, Tas.	R. Plapp	Erumpent
54/2	West Pine, Tas.	B. Bott	Erumpent
54/3	West Pine, Tas.	B. Bott	Erumpent
55/2	Upper Castra, Tas.	M. Whitley	Pitted
56/2	Upper Castra, Tas.	M. Whitley	Russet
57/1	Riana, Tas.	D. Norris	Russet
57/1-2	Riana, Tas.	D. Norris	Russet
59/1	Moriarty, Tas.	D. Addison	Russet
62/1	Sprent, Tas.	H. Morris	Russet
62/1-2	Sprent, Tas.	H. Morris	Russet
63/1	South Riana, Tas.	W. Fielding	Erumpent
64/1	West Pine, Tas.	R. Plapp	Russet
68/1	West Pine, Tas.	R. Plapp	Erumpent
72/1-1	Barrington, Tas.	I. Smith	Erumpent
72/1-2	Barrington, Tas.	I. Smith	Erumpent
72/1-3	Barrington, Tas.	I. Smith	Erumpent
73/1-1	West Pine, Tas.	R. Plapp	Erumpent
73/1-2	West Pine, Tas.	R. Plapp	Erumpent

....continued next page

Table 4.1 (continued)

Strain	Source Site	Grower	Lesion Type
73/1-4	West Pine, Tas.	R. Plapp	Erumpent
73/1-5	West Pine, Tas.	R. Plapp	Erumpent
75/1-1	Riana, Tas.	D. Norris	Russet
76/1-1	West Pine, Tas.	L. Plapp	Erumpent
76/1-2	West Pine, Tas.	L. Plapp	Erumpent
77/1	West Pine, Tas.	L. Plapp	Erumpent
77/3	West Pine, Tas.	L. Plapp	Erumpent
79/2-1	Moriarty, Tas.	N. Badcock	Russet
79/2-3	Moriarty, Tas.	N. Badcock	Russet
80/2-1	Barrington, Tas.	D. Brown	Erumpent
85/1	Penguin, Tas.	G. McKenna	Pitted
86/1	Forth, Tas.	B. Hopkins	Pitted
88/1	Sprent, Tas.	H. Morris	Pitted
93/2	West Pine, Tas.	L. Plapp	Erumpent
94/1-1	West Pine, Tas.	L. Plapp	Erumpent
94/1-2	West Pine, Tas.	L. Plapp	Erumpent
95/1	West Pine, Tas.	R. Plapp	Russet
95/2-1	West Pine, Tas.	R. Plapp	Russet
95/2-2	West Pine, Tas.	R. Plapp	Russet
98/1	South Riana, Tas.	D. Carr	Erumpent
105/1-1	West Pine, Tas.	L. Plapp	Russet
105/1-2	West Pine, Tas.	L. Plapp	Russet
105/2	West Pine, Tas.	L. Plapp	Erumpent
106/1	Barrington, Tas.	C. Fitzmaurice	Erumpent
108/2	Barrington, Tas.	D. Brown	Erumpent

[1] Cultivar had not been recorded at the time of collection but in the majority of cases was probably Russet Burbank as this was the predominant cultivar grown.

Table 4.2 Strains obtained from M. Stroman of DPIWE [1]

Strain	Source Site	Grower	Lesion Type
1/1	Waterhouse, Tas.	K. Lette	Russet
5/1	South Riana, Tas.	A. Clarke	Erumpent
6/1B	Branxholm, Tas.	J. Bennett	Lenticel
8/2	Forth, Tas.	FVRS[2]	Russet
9/1	Forth, Tas.	FVRS	Russet
12/1A	Forth, Tas.	FVRS	Lenticel
12/2A	Forth, Tas.	FVRS	Russet
12/2B	Forth, Tas.	FVRS	Russet
13/1B	Forth, Tas.	FVRS	Russet
14/1A	Forth, Tas.	FVRS	Russet
15/1B	Forth, Tas.	FVRS	Russet
15/2A	Forth, Tas.	FVRS	Russet
15/3A	Forth, Tas.	FVRS	Russet
16/2	Forth, Tas.	FVRS	Russet
16/2-2	Forth, Tas.	FVRS	Russet
18/2B	Forth, Tas.	FVRS	Russet
19/1A-1	Forth, Tas.	FVRS	Russet
19/1A-2	Forth, Tas.	FVRS	Russet
19/1B	Forth, Tas.	FVRS	Russet
20/1A	Forth, Tas.	FVRS	Pitted
20/1B	Forth, Tas.	FVRS	Pitted
95/13/1A[4]	Burnie, Tas.	n.d.[3]	n.d.
#23[5]	Tasmania	n.d.	n.d.
#25[5]	Tasmania	n.d.	n.d.
#27[5]	Tasmania	n.d.	n.d.
#32[5]	Victoria	n.d.	n.d.

[1] From tubers collected in the 1995 scab survey unless otherwise noted. In the majority of cases potato cultivar was probably Russet Burbank as this was the predominant cultivar grown. Cultivar had not been recorded at the time of collection.

[2] FVRS = Forthside Vegetable Research Station.

[3] n.d. = Not determined.

[4] Isolate from 1994 scab survey

[5] Strains from a previous DPIWE collection by C. Gilliam.

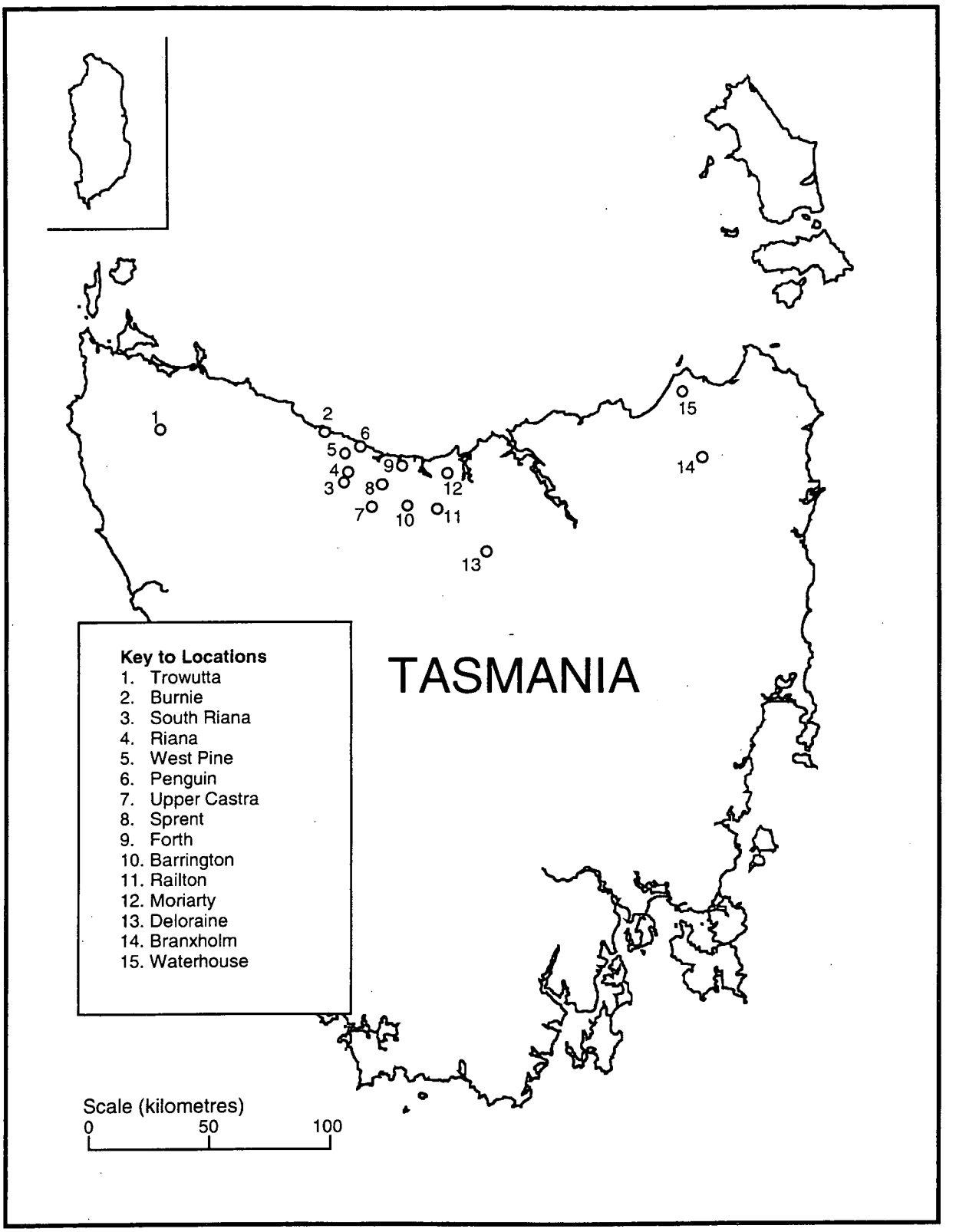
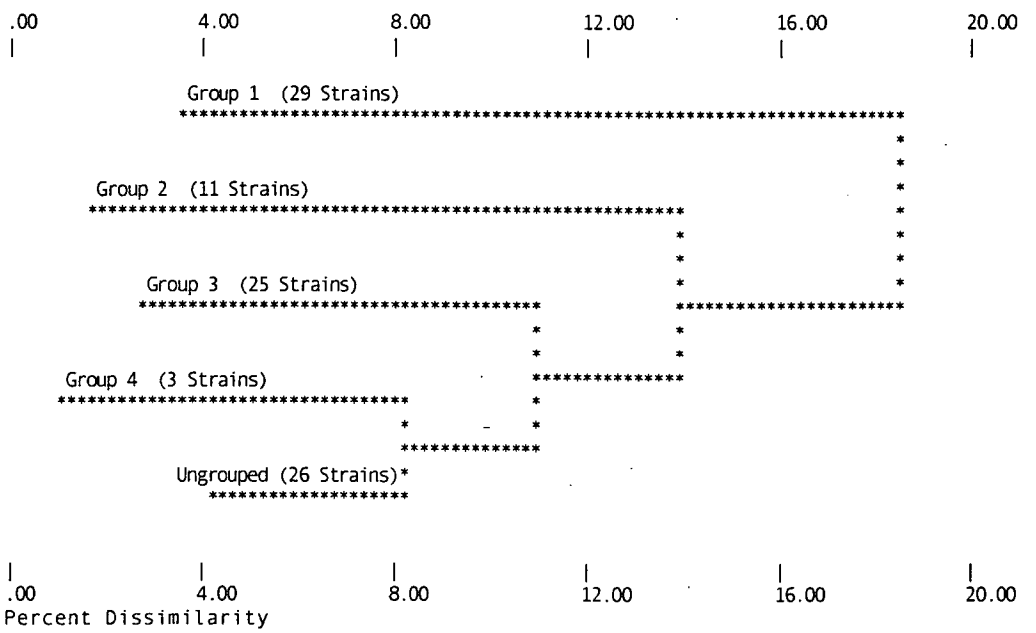


Figure 4.3 Map showing known source locations of tubers from which strains were isolated

4.3.2 Strain Characteristics

Morphological and physiological characteristics were determined for 94 strains (listed in Appendix 2.1). Strains were readily sorted into five groups (Figure 4.4) based on Burr's strategy (Ross and Shields, 1993). Characteristics used in sorting are listed in Appendix 2.2 and included 15 binary, three multistate and five numeric traits.

Figure 4.4 Grouping of strains according to Taxon 1.0 using Burr's sorting strategy.



Strains included in groups:

Group 1: 2/1-4, 2/1-5, 2/2-4, 2/2-5, 42/1-2, 42/2-1, 43/1-3, 44/1A-1, 46/1B-1, 51/2-1, 52/2, 57/1, 63/1, 72/1-3, 73/1-1, 73/1-4, 76/1-1, 76/1-2, 77/1, 88/1, 93/2, 95/1, 98/1, 105/1-1, 105/1-2, 105/2, 106/1, 108/2, #25.

Group 2: 43/1-2, 46/1A-1, 46/2B, 48/2, 49/1, 54/2, 54/3, 62/1, 62/1-2, 75/1-1, 94/1-2.

Group 3: 1/1, 1/2-3, 2/2-3, 8/2, 9/1, 12/1A, 12/2A-1, 12/2B, 13/1B, 14/1A, 15/1B, 15/2A, 15/3A, 16/2, 16/2-2, 18/2B, 19/1A-2, 19/1B, 55/2, 56/2, 57/1-2, 94/1-1, #23, #32, 95-13-1A.

Group 4: 68/1, 72/1-2, 95/2-1.

Ungrouped: 1/2-4, 2/1-1, 2/1-3, 2/2-2, 5/1, 6/1B, 19/1A-1, 20/1A, 20/1B, 25/2, 41/2, 43/1-1, 51/1, 53/2, 59/1, 64/1, 72/1-1, 73/1-2, 73/1-5, 77/3, 79/2-1, 79/2-3, 80/2-1, 85/1, 86/1, 95/2-2.

Morphological and physiological characteristics of strains in the five groups are summarised in Table 4.3. Most strains were identified as *Streptomyces* species, with the exception of three strains producing globular sporangia, identified as *Streptosporangium* spp. (Group 4). Description of strains in each group and species designations are as follows:

Group 1 consisted of 29 strains which predominantly produced grey spores in flexuous chains, grew with 7% NaCl and had a minimum pH for growth of 6.0. Inositol was not used as sole carbon source by any of these strains, while raffinose was used by only 17%. Melanin was not produced but a pale yellow pigment was produced by 60% of strains. Substrate mycelia tended to be yellowish, especially in young cultures, and spore mass had a powdery texture. Characteristics of members of this group most closely fitted the probabilistic description of *S. halstedii*, differing in production of diffusible pigment which is usually absent for this species (Williams *et al.*, 1983a).

Group 2 consisted of 11 strains which produced grey spores in tight spirals, did not produce melanin, used all of the ISP sugars and did not grow with 7% NaCl. A very pale orange diffusible pigment was discernible in agar media. Starch was degraded by only 27% of strains and xanthine by 18%. The pH minimum for most strains was 5.5. Plate cultures were hygroscopic, resulting in the spore mass disintegrating to a mucoid black liquid after approximately one month. This group fitted the description of *S. violaceusniger* (Williams *et al.* 1983a)

Group 3 consisted of 25 strains with characteristics consistent with *S. scabies* (Lambert and Loria, 1989a) ie. strains had grey-brown (or grey) spores borne in spiral chains, melanin was produced and all ISP sugars were used by the majority of strains. This group included the reference strain #32. These strains differed from the descriptions of Lambert and Loria (1989) in growing at a pH lower than 5.0. All strains grew at pH 4.5 or lower, with 20% growing at pH 4.0. These strains also differed from type strains in degrading xanthine and growing in the presence of 0.5 µg/ml crystal violet, 0.1% phenol, 10 IU penicillin-G or 100 µl/ml oleandomycin. With the exception of two strains producing grey spores in loose spirals, all produced grey-brown spores in tight spirals.

Group 4 consisted of three *Streptosporangium* strains. These strains produced pink to orange spores in globular sporangia; grew with

20 µl/ml streptomycin, did not use D-mannitol, D-raffinose or meso-inositol; did not grow with 5% NaCl; did not degrade xanthine; and had a minimum pH for growth of 6.5.

Ungrouped. Twenty six strains did not clearly fit into any of the other groups although many of these had characteristics resembling Groups 1 or 3. Of 13 strains which produced melanin, most produced grey spores in loose spiral chains and all-but-two did not degrade xanthine, and in these respects these strains more closely fitted the classical description of *S. scabies*. Five strains in this melanin producing sub-group did not use mannitol.

All strains used L-arabinose, D-glucose, D-xylose, L-hydroxyproline and L-methionine and grew in the presence of 10 IU/ml penicillin G. The majority of strains, with the exception of some strains in Group 1, did not grow with 100 µg/ml tellurite or 100 µg/ml thallium.

Table 4.3 Characteristics of five groups determined by Taxon using Burr's strategy.

Strain group	**1**	**2**	**3**	**4**	**Ungrouped**
Number of strains	29	11	25	3	26
Characteristic					
Spore colour on YME	Grey (86%)[§] White (14%)	Grey	Grey-Brown (92%) Grey (8%)	Pink	Grey (85%) Grey-Brown (8%) White (8%)
Hygroscopic cultures[#]	—	+	—	—	—
Spore bearing structures	Flexuous chains (97%) Loose-Spiral chains (3%)	Tight-Spiral chains	Tight-Spiral chains (92%) Loose-Spiral chains (8%)	Globular Sporangia	Tight-Spiral chains (12%) Loose-Spiral chains (65%) Flexuous chains (23%)
Melanin on tyrosine agar[#]	—	—	+	—	50
Melanin on PYI[#]	—	—	+	—	50
Diffusible pigment on GAT	Yellow (64%) Nil (46%)	Pale orange	Nil	Nil	Nil (80%) Yellow (20%)
Carbon usage[#]					
L-Arabinose	+	+	+	+	+
D-Fructose	+	+	+	33	92
D-Glucose	+	+	+	+	+
D-Mannitol	97	+	+	—	65
D-Raffinose	17	+	96	—	85
α-L-Rhamnose	66	+	+	+	96
Sucrose	83	+	88	67	+
D-Xylose	+	+	+	+	+
meso-Inositol	—	+	+	—	92
Nitrogen usage[#]					
L-Hydroxyproline	+	+	+	+	+
L-Methionine	+	+	+	+	+
Degradation of:[#]					
Arbutin	+	+	¶ (92%) + (8%)	+	¶ (44%) + (56%)
Polygalacturonate	86	+	+	—	75
Xanthine	97	18	+	—	27
Xylan	97	+	72	—	73
Starch	+	27	+	+	96
Minimum Growth pH	6.5 (3%) 6.0 (41%) 5.5 (55%)	5.5 (73%) 5.0 (27%)	4.5 (80%) 4.0 (20%)	6.5 (100%)	6.0 (15%) 5.5 (23%) 5.0 (12%) 4.5 (50%)

Table continued next page...

Table 4.3 continued.

Strain group	1	2	3	4	Ungrouped
Characteristic					
Growth with: [#]					
5%NaCl	+	73	+	—	42
6%NaCl	+	36	56	—	23
7%NaCl	93	—	24	—	23
Tellurite (10µg/ml)	+	45	80	+	54
Tellurite (100µg/ml)	7	—	—	—	—
Thallium (10µg/ml)	59	9	56	67	8
Thallium (100µg/ml)	3	—	—	—	—
Crystal Violet (0.5µg/ml)	93	82	96	67	92
Phenol (0.1%)	97	18	+	+	81
Penicillin (10 IU/ml)	+	+	+	+	+
Oleandomycin (25µg/ml)	97	+	76	+	46
Oleandomycin (100µg/ml)	79	73	56	+	31
Streptomycin (20µg/ml)	3	18	—	+	—

[§] Figures in parentheses show percentage of strains with characteristic.

[#] + positive;— negative; otherwise figures show percentage of positive strains in group.

¶ Result not determined (reaction obscured by pigment)

4.3.3 Pathogenicity tests

4.3.3.1 Potato disk assay

Although results of the potato disk assay varied to some extent between replicates a general consensus could be determined for each strain. Strains could be broadly classified into three groups:

1) Strains which did not grow on or cause any apparent change in the tuber disk (eg. Figure 4.5, C). Necrosis (brown discolouration) was absent or very superficial and confined to where the agar block had been in contact with the potato tissue . Sporulation, if any, was very sparse and mostly confined to the agar block. This was designated a nil reaction

2) Strains which grew vigorously on the tuber slice (eg. Figure 4.5, A). Necrosis and softening of the potato tissue to the full depth of the slice. This was designated a pathogenic reaction

3) Strains which grew on the potato disk to some extent, with limited necrosis underneath and immediately around the agar block, not extending as deeply as observed for the second group (eg. Figure 4.5, B). Growth of the strain on the potato disk was mainly close to the agar block. Designated a weak reaction.

Table 4.4 summarises reaction type sorted by taxonomic grouping. Strains showing the pathogenic reaction included: all of the *S. violaceusniger* strains; the *S. scabies* reference strain #32 and; five (19%) of the ungrouped strains. Most (84%) of the *S. scabies* strains showed a weak reaction, as did a large proportion of the *S. halstedii*-like and ungrouped strains. All three *Streptosporangium* strains showed no sign of pathogenicity in this assay.

The five ungrouped strains showing a pathogenic reaction resembled *S. scabies*. These strains: produced grey spores in spiral chains; used all of the ISP sugars (with the exception of strain 2/1-3 which did not use mannitol); did not degrade xanthine and; did not grow with 5% NaCl (except strain 2/1-3 which grew at 5% but not 6% NaCl). Four of these strains produced melanin. Minimum pH for growth ranged between 4.0 and 6.0.

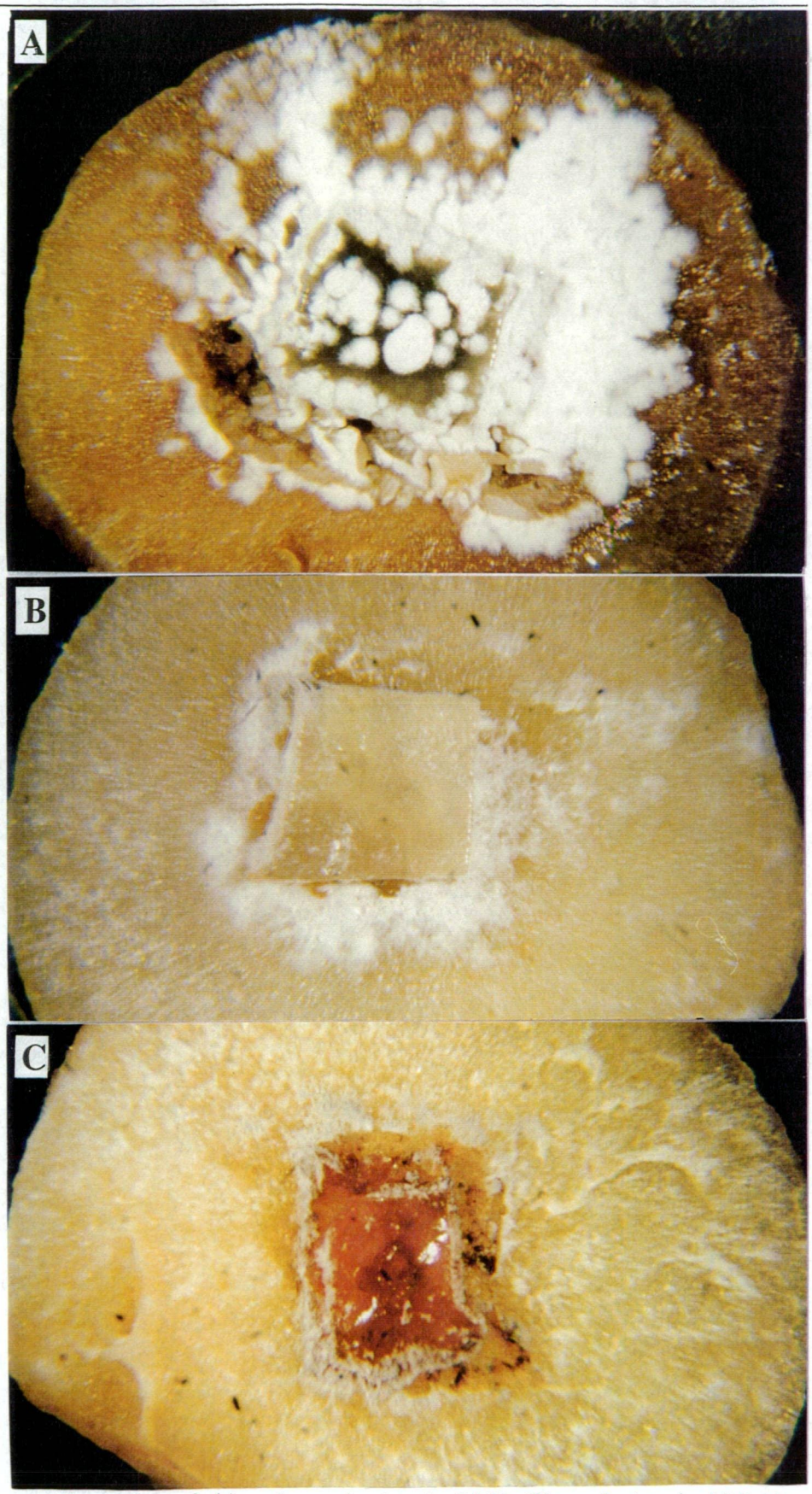


Figure 4.5 Examples of: **A)** pathogenic, (strain 54/3); **B)** weak, (strain 12/1A); **C)** nil, (strain 80/2-1) reactions on potato disks of cultivar Bismark at six days after applying oatmeal agar inoculum to the slice.

Table 4.4 Pathogenicity reaction of strains in tuber slice assay, listed by taxonomic group[#]

Reaction \ Strain group	"Pathogenic" reaction	Weak to nil reaction	Nil reaction
Group 1 (*Streptomyces halstedii*-like)	None	22 strains (76%) 2/1-4, 2/2-5, 42/1-2, 42/2-1, 43/1-3, 44/1A-1, 46/1B-1, 57/1, 63/1, 73/1-1, 73/1-4, 76/1-1, 76/1-2, 77/1, 88/1, 93/2, 95/1, 98/1, 105/1-1, 105/1-2, 105/2, 108/2.	7 strains (24%) 2/1-5, 2/2-4, 51/2-1,52/2, 72/1-3, 106/1, #25.
Group 2 (*Streptomyces violaceusniger*)	11 strains (100%) 43/1-2, 46/1A-1, 49/1, 46/2B, 48/2, 54/3, 54/2, 62/1, 62/1-2, 75/1-1, 94/1-2.	None	None
Group 3 (*Streptomyces scabies*)	1 strain (4%) #32.	21 strains (84%) 1/1, 1/2-3, 8/2, 9/1, 12/1A, 12/2A-1, 12/2B, 13/1B, 14/1A, 15/1B, 15/2A, 15/3A, 16/2, 16/2-2, 18/2B, 19/1A-2, 19/1B, 57/1-2, 94/1-1, #23, 95-13-1A.	3 strains (12%) 2/2-3, 55/2, 56/2.
Group 4 (*Strepto-sporangium* spp.)	None	None	3 strains (100%) 68/1, 72/1-2, 95/2-1.
Ungrouped	5 strains (19%) 2/1-1, 2/1-3, 53/2, 73/1-2, 95/2-2.	13 strains (50%) 2/2-2, 5/1, 6/1B, 20/1A, 20/1B, 41/2, 43/1-1, 73/1-5, 59/1, 64/1, 72/1-1, 80/2-1, 85/1.	8 strains (31%) 1/2-4, 19/1A-1, 25/2, 51/1, 77/3, 79/2-1, 79/2-3, 86/1.

[#] Table entries show: number of strains in group with reaction, (percentage for group in brackets) followed by listing of strains affected.

4.3.3.2 Radish seedling assay

A significant reduction in shoot growth was observed following inoculation of radish seedlings with 0.125 ml of culture supernatant from either of ten *S. scabies* strains, eight out of ten *S. violaceusniger* strains or strain 25/2 (ungrouped) compared to uninoculated controls (Figures 4.6 and 4.7). Root hair development was also inhibited or absent from affected seedlings. Media from two of the *S. violaceusniger* strains (54/3 and 75/1-1) also promoted complete necrosis of seedlings within one day of application. Little or no effect on seedling growth was produced by supernatant from any of three *S. halstedii*-like strains or two of three ungrouped strains tested.

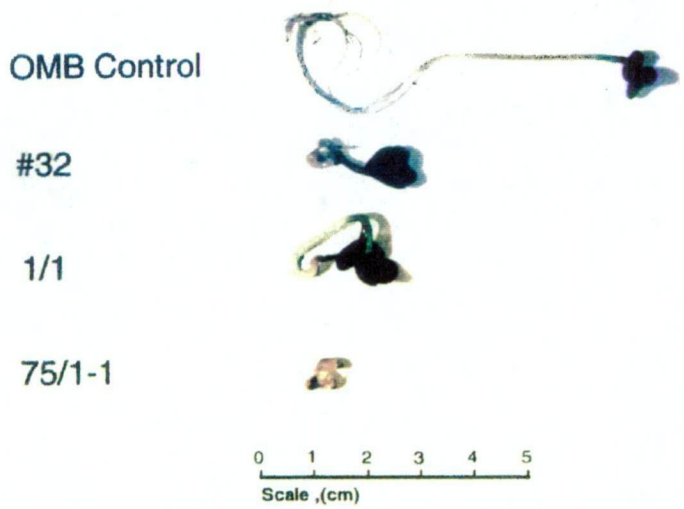


Figure 4.6 Radish seedlings, nine days after inoculation of pre-germinated seeds with 0.125 ml of supernatant from: uninoculated oatmeal broth (OMB) or; OMB cultures of *S. scabies* strain #32, 1/1 or *S. violaceusniger* strain 75/1-1.

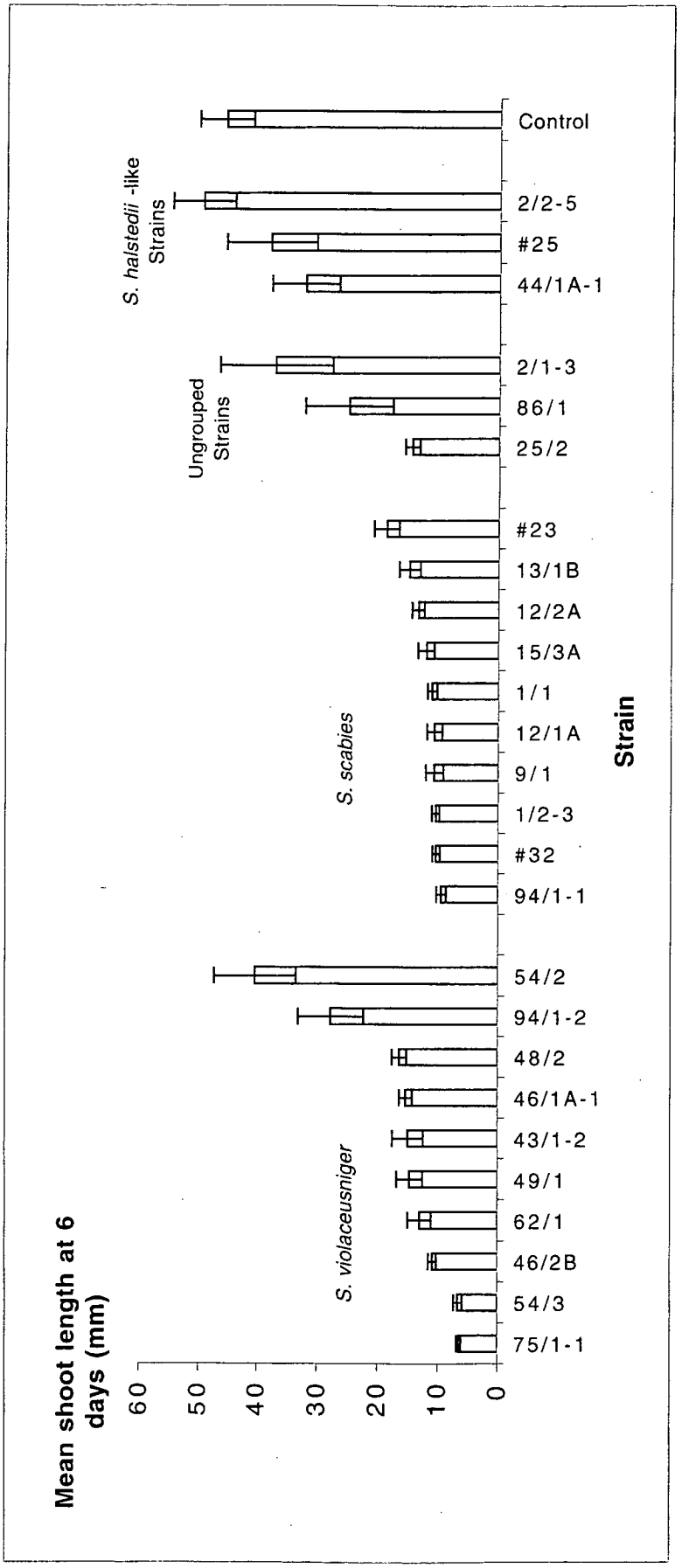


Figure 4.7 Mean shoot length of radish seedlings at six days after inoculation of pre-germinated seeds with 0.125 ml of supernatant from oatmeal broth cultures of either of 26 *Streptomyces* strains or uninoculated broth control. Error bars show standard error. N = 6.

4.3.3.3 Minituber assay

Pathogenicity, of 26 strains was re-assessed on potato minitubers. Scab severity scores for tubers assessed as either severe, moderate, slight or nil and are shown in Table 4.5, with results of the potato disk and radish seedling assays included for comparison.

Necrosis, where observed, was mainly confined to lenticels (Figure 4.8). No necrosis was found on negative controls (Figure 4.10 'OMB Control'). For *S. scabies* strains there was either no effect (7 strains) or a slight darkening of lenticels (3 strains; eg. Figure 4.10 '1/2-3'). Culture broth from *S. violaceusniger* strains caused complete necrosis of green leafy tissue within one day of application (visible in Figures 4.8, 4.9. and 4.10). This was particularly evident with strains 54/3 and 75/1-1 which also caused extensive necrosis of tuber surfaces (Figures 4.8 and 4.10 '75/1-1'). Representatives of the *S. halstedii*-like strains and selected ungrouped strains produced no observable effect on minitubers.

Figure 4.8 Potato stem minituber at 4 days following immersion for one minute in supernatant from oatmeal broth culture of strain 46/1A-1.

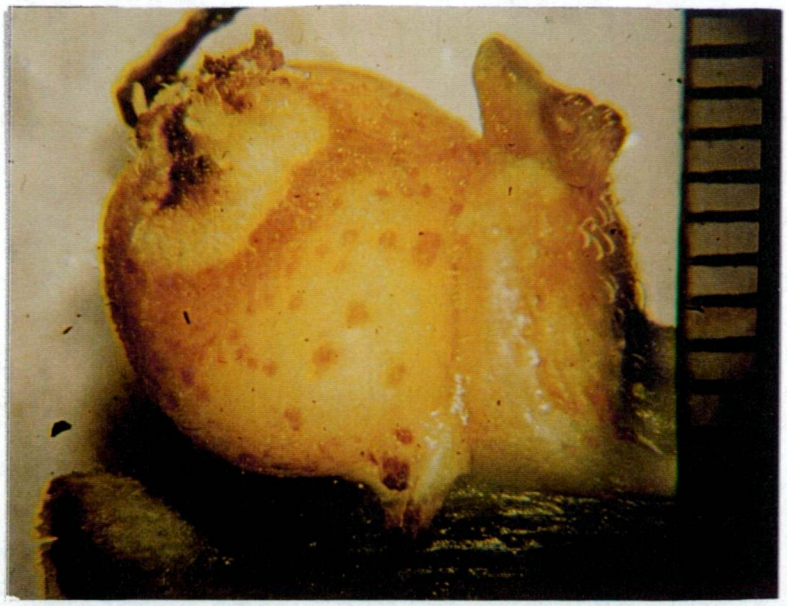


Figure 4.9 Potato stem minituber at 4 days following immersion for one minute in supernatant from oatmeal broth culture of strain 75/1-1.

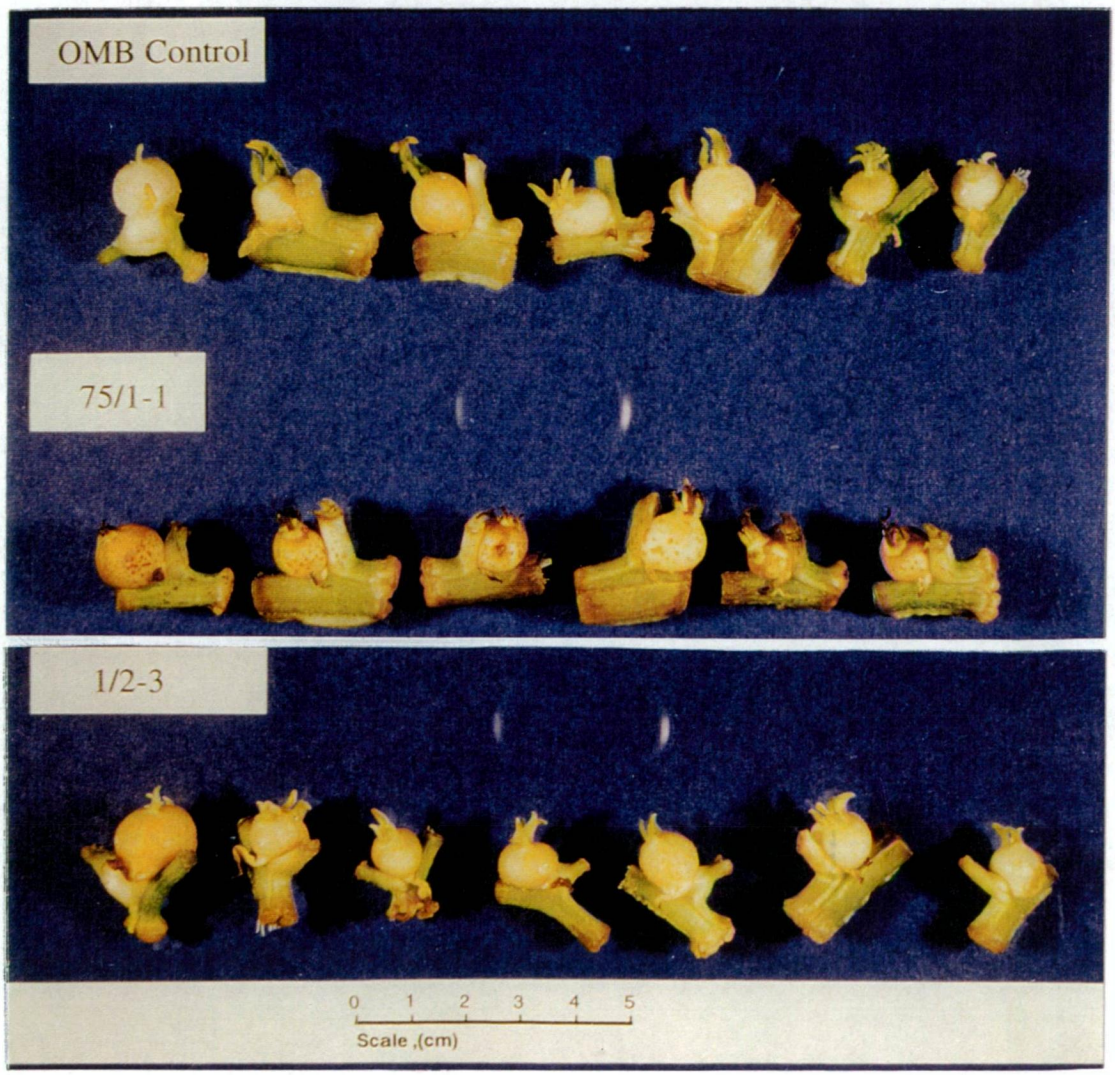


Figure 4.10 Potato stem minitubers at 4 days following immersion for one minute in supernatant from: uninoculated oatmeal broth (OMB Control) or; oatmeal broth culture of *S. violaceusniger* strain 75/1-1 or *S. scabies* strain 1/2-3.

Table 4.5 Comparison of results of pathogenicity assays for 26 strains

Strain	Strain group	Tuber disk assay [1]	Radish seedling assay [2]	Minituber assay [3]
			Shoot length, (mm)	Severity of necrosis
75/1-1	*S. violaceusniger*	++	6.4 ± 0.4	++++
54/3	*S. violaceusniger*	++	6.6 ± 0.7	++++
46/2B	*S. violaceusniger*	++	10.8 ± 0.7	++
62/1	*S. violaceusniger*	++	12.9 ± 1.9	++
49/1	*S. violaceusniger*	++	14.5 ± 2.1	++
43/1-2	*S. violaceusniger*	++	14.8 ± 2.5	++
46/1A-1	*S. violaceusniger*	++	15.2 ± 1.0	++
48/2	*S. violaceusniger*	++	16.2 ± 1.2	++
94/1-2	*S. violaceusniger*	++	27.7 ± 5.4	++
54/2	*S. violaceusniger*	++	40.3 ± 6.9	++
94/1-1	*S. scabies*	+	9.5 ± 0.8	–
#32	*S. scabies*	++	10.3 ± 0.6	+
1/2-3	*S. scabies*	+	10.4 ± 0.6	+
9/1	*S. scabies*	+	10.6 ± 1.5	–
12/1A	*S. scabies*	+	10.6 ± 1.3	+
1/1	*S. scabies*	+	11.0 ± 0.8	–
15/3A	*S. scabies*	+	12.0 ± 1.3	–
12/2A	*S. scabies*	+	13.3 ± 1.0	–
13/1B	*S. scabies*	+	14.7 ± 1.7	–
#23	*S. scabies*	+	18.5 ± 2.1	–
25/2	Ungrouped	–	14.3 ± 1.1	–
86/1	Ungrouped	–	24.8 ± 7.3	–
2/1-3	Ungrouped	++	37.0 ± 9.4	–
44/1A-1	*S. halstedii*-like	+	32.1 ± 5.6	–
#25	*S. halstedii*-like	–	37.8 ± 7.6	–
2/2-5	*S. halstedii*-like	+	49.3 ± 5.3	–
Control	N.A.	–	45.5 ± 4.6	–

[1] Tuber disk assay: ++, "pathogen"; +, weak to nil ; -, nil.

[2] Radish seedling assay: figures show mean shoot length at 6 days ± standard error, (n=6).

[3] Minituber assay. Necrosis severity : ++++, severe ; ++, moderate ; +, slight ; –, nil.

4.3.3.4 Pot trial

Pathogenicity of four *S. scabies* and four *S. violaceusniger* strains was assessed on potatoes growing in sand. Scab severity was very mild, indicating that conditions were sub-optimal for scab formation. Some atypical scab-like lesions were recorded for four strains including the positive control (*S. scabies* strain #32), one other *S. scabies* strain (13/1B) and two *S. violaceusniger* strains (48/2 and 54/3). No scab was observed for the un-inoculated control, or the four other strains tested, which included three *S. scabies* strains (1/2-3, 9/1, 12/1A) and one *S. violaceusniger* strain (46/1A-1). No actinomycetes were found amongst isolates from tuber lesions. Pathogenicity of any strain tested in this pot trial was thus not confirmed, as lesions were mild and atypical and Koch's postulates were not fulfilled.

4.3.4 Analysis of culture extracts

OMB culture supernatants of 18 strains which had previously been used in radish seedling and minituber assays were extracted with chloroform following five months storage at -20°C. Colour of the final extracts were: *S scabies* strains, pale yellow; *S. violaceusniger* strains, mostly colourless or very pale yellow, except for strains 54/3 and 75/1-1 which were intense yellow; strain 25/2, pale yellow; strain 2/1-3, orange; uninoculated OMB media, colourless (Figure 4.11).

Similar extracts were prepared from fresh cultures of; *S. scabies* strains #32, 12/1A, 1/1; *S. violaceusniger* strain 54/3 and *S. scabies*-like ungrouped strain 85/1.

It was noted that although culture supernatants for *S. scabies* strains had a pale yellow colour, this colour predominantly remained in the aqueous phase instead of being transferred to the chloroform phase.

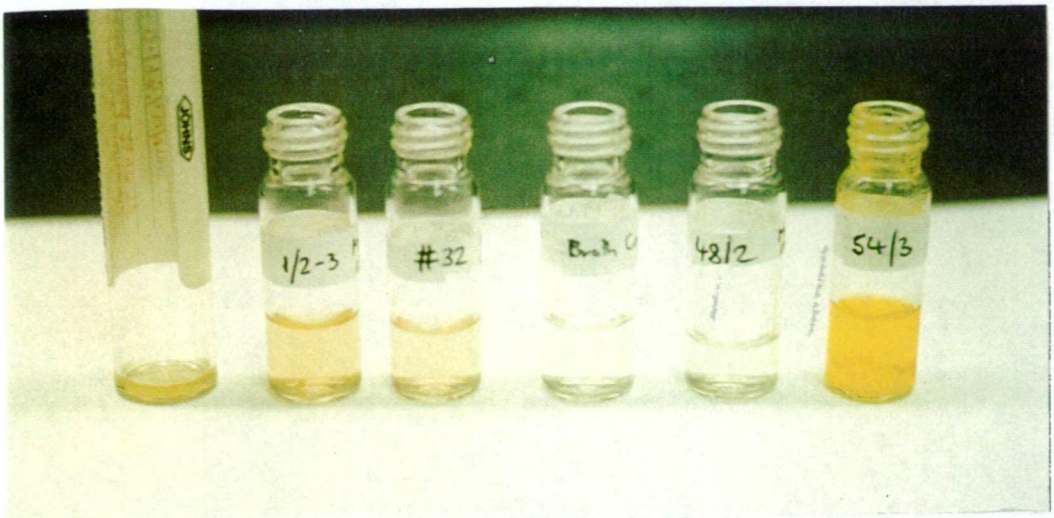


Figure 4.11 Extracts dissolved in methanol (from left to right): authentic thaxtomin A; chloroform extracts of OMB broth cultures for, *S. scabies* strains 1/2-3 and #32, uninoculated control, *S. violaceusniger* strains 48/2 and 54/3.

4.3.4.1 Thin-layer chromatography

4.3.4.1.1 TLC of stored extracts

Chloroform extracts from OMB cultures of eight *S. scabies* strains, eight *S. violaceusniger* strains, two of the ungrouped strains (2/1-3, and 25/2) and a sample of authentic thaxtomin A were compared by TLC. Examination of this plate under 254 nm UV light (Figure 4.12) revealed that the *S. scabies* strains (including the reference strain #32) shared an almost identical pattern of bands, which indicated that these strains represented a uniform group. Lack of a clear band corresponding to thaxtomin A, indicated that thaxtomin A was not present at a detectable concentration in these samples.

TLC band patterns for *S. violaceusniger* strains resembled one another and were different to those of *S. scabies*. The bands for strain 54/3 were fainter than for other *S. violaceusniger* strains due to a smaller amount of extract being applied to the plate of which the main constituent moved in an intense yellow band close to the advancing front. This yellow band was extracted from a separate plate for analysis by mass spectrometry.

Observation of fluorescence under 366 nm light (Figure 4.13) further confirmed similarities within the two respective groups noted above and of the differences between them. A fluorescent band corresponding to one visible at Rf 0.64 for strain 48/2 on this plate and shared by the *S. violaceusniger* strains was extracted for analysis by mass spectrometry.

Bands for strains 2/1-3 and 25/2 visible at both 254 and 366 nm appeared similar to those for *S. scabies* strains indicating that these could also be *S. scabies*.

4.3.4.1.2 TLC of extracts from fresh OMB cultures

In a TLC assay of extracts from fresh OMB cultures, thaxtomin A was detected in media from *S. scabies* strain #32 but not *S. scabies* strains 12/1A or 1/1, *S. violaceusniger* strain 54/3 or *S. scabies*-like ungrouped strain 85/1.

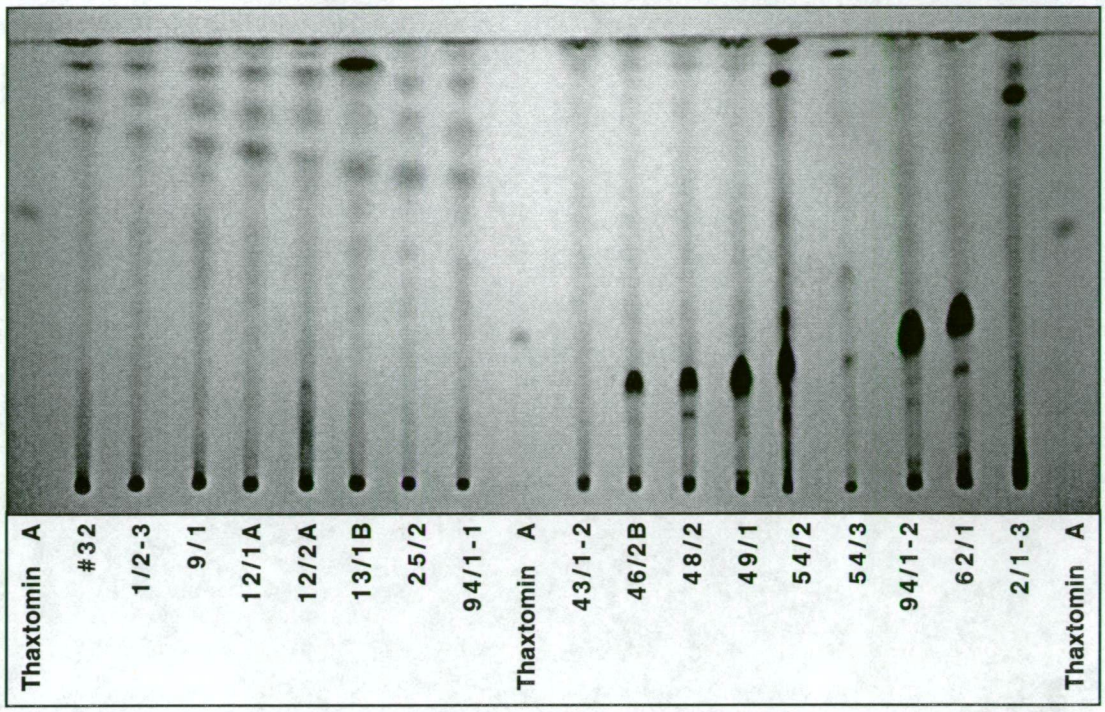


Figure 4.12 TLC plate of chloroform extracts from OMB cultures of 17 strains showing bands visible under 254 nm UV light. Authentic thaxtomin A is included in lanes 1, 10 and 20.

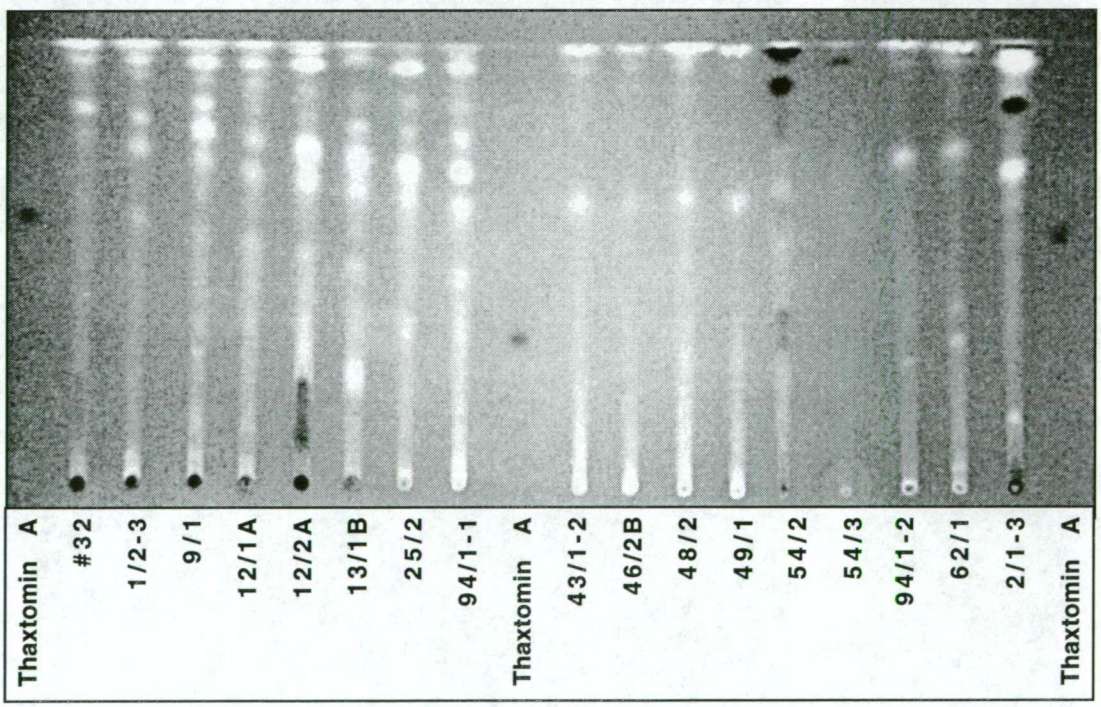


Figure 4.13 The same TLC plate showing fluorescence (pale areas) visible under 366 nm UV light.

4.3.4.2 Mass spectrometric analyses

Chloroform extracts from oatmeal broth cultures used in radish seedling and minituber assays were examined by mass spectrometry (MS). Details of MS analyses are presented below with some discussion (for simplicity of presentation).

4.3.4.2.1 Evidence for absence of thaxtomin A in extracts

Initially the presence of thaxtomin A in extracts for strains #32, 1/2-3, and 75/1-1 was assessed using the Kratos Concept ISQ in electron ionisation (EI) mode. The EI mass spectrum obtained for authentic thaxtomin A matched published data (King *et al.*, 1992), and included fragments at m/e 175, 162, 116, 107, and the molecular ion (M) at m/e 438. Analysis of the three extracts revealed no evidence of thaxtomin A as determined by absence of the fragments noted above and in particular the absence of a fragment at m/e 162.0429 when examined at high resolution.

With the extracts being essentially a mixture of compounds it was considered that HPLC separation into components before MS analysis would aid more precise measurement. Hence, culture extracts of two representative *S. scabies* strains (1/2-3 and #32) and two *S. violaceusniger* strains (46/2B and 54/3) were analysed by HPLC-MS using the Finnigan LCQ. A thaxtomin screening procedure was devised as follows: 5µl of sample was injected and run, with the HPLC mobile phase consisting of 50/50 methanol/water for 8 min then to 100 methanol at 20 mins then to 80/20 methanol/hexane at 30 min. Using this procedure, thaxtomin A produced a HPLC peak at 7.0 minutes with the APCI MS spectra showing main ions at m/e 420 (Figure 4.14). Assessment of the four extracts revealed no corresponding HPLC peak at around 7 minutes, indicating the absence of thaxtomin A from these samples (Figure 4.14).

Together with evidence from TLC analysis of extracts, this MS data indicates the absence of measurable quantities of thaxtomin A from the extracts at the time of measurement.

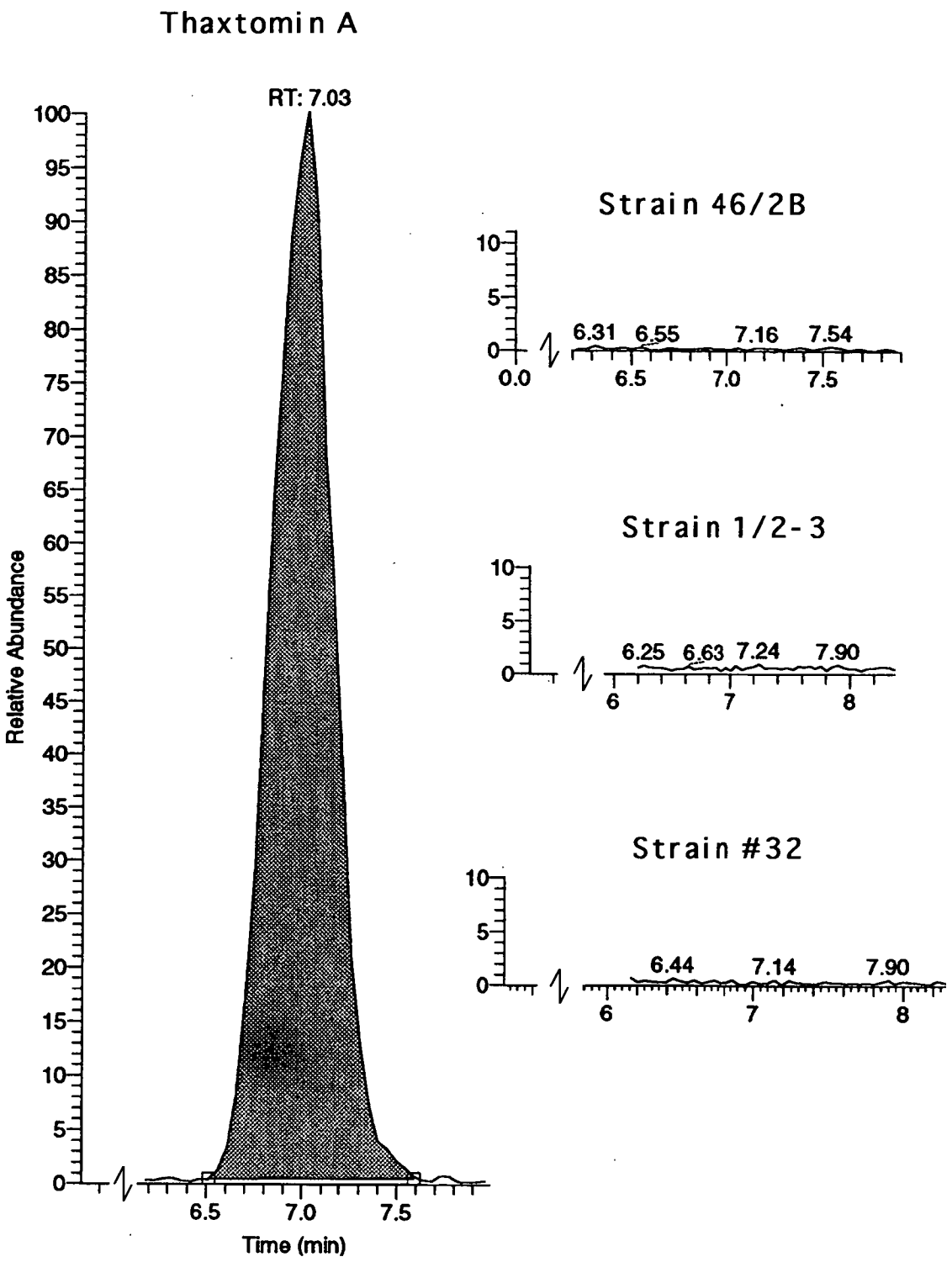


Figure 4.14 HPLC-MS thaxtomin screen. Charts show relative abundance of fragments in the range m/e 420.5 to 421.5 at around seven minutes, for authentic thaxtomin A; and chloroform extracts of supernatant from oatmeal broth cultures of strains 46/2B, 1/2-3, and #32. Also (not shown) corresponding measurements for strain 54/3 were similar to strain 46/2B.

4.3.4.2.2 Elucidation of the likely identity of observed diffusible metabololites from *S. violaceusniger* strains

Using the thaxtomin screening procedure, additional non-thaxtomin compounds were detected in the *S. violaceusniger* extracts. A very large HPLC peak with a retention time of 17.1 minutes was observed for 54/3 (Figure 4.15). A UV spectrum was obtained for this peak (Figure 4.16). A group of smaller HPLC peaks between 21 and 22.4 minutes were shared by extracts 54/3 and 46/2B. The larger of these peaks did not absorb in the UV range.

Further investigations were carried out to determine the identity of the compounds found in extracts from *S. violaceusniger* strains.

It was known that the main constituent of extracts from strains 54/3 and 75/1-1 was a yellow substance that formed orange/yellow needle-shaped crystals on evaporation of a methanol solution. This substance was also much more soluble in chloroform than methanol. EI mass spectra of extracts (eg. Figure 4.17; 54/3) from these strains revealed that they were composed of an almost pure substance. It was, therefore, considered probable that the large HPLC peak observed for strain 54/3 (Figure 4.15) was the same substance. A sample of the yellow material abundantly produced by strain 54/3 was separated out by TLC and assessed by electrospray MS. This sample gave a prominent peak at 583 (M + sodium) and also at 1143 (2M+sodium) indicating a molecular weight of 560 for the major component (Figure 4.18). However, the molecular weight was too large to determine molecular formula from MS data.

A minor component of extracts from strains 54/3 and 75/1-1 formed colourless crystals, as did the extracts from all other *S. violaceusniger* strains.

In the TLC comparison of culture extracts a fluorescent band, (visible with 366nm UV light) was common to all *S. violaceusniger* strains (Figure 4.13). This band was extracted from a TLC plate for strain 48/2 and analysed by ESI MS-MS by direct infusion. The MS spectrum revealed that the major component had an apparent molecular weight of 746 as indicated by peaks at 747 (M + H?) and 769 (M

+sodium?). MS-MS fragmentation patterns were generated for the 747 peak (Figure 4.19).

At this stage there was evidence for a yellow compound with molecular weight of 560 and one or more colourless compounds including one with molecular weight of around 746. Molecular weights of these compounds were too large to determine structural formulae from precise weight determinations. It was believed that some of these compounds may have been responsible for symptoms observed in pathogenicity assays. A search of literature for candidate compounds ensued, with particular emphasis on known metabolites of *S. violaceusniger* and related species. Strains of *S. hygroscopicus* have been grouped with *S. violaceusniger* in recent taxonomic studies (Kampfer *et al.*, 1991; Williams *et al.* 1983b). A metabolite of molecular weight 560 (geldanamycin) produced by a strain of *S. hygroscopicus* and another compound (nigericin, MW 725) produced by the same strain have been shown to have phytotoxic properties (Heisey and Putnam, 1990).

4.3.4.2.3 Evidence for presence of geldanamycin in extracts of *S. violaceusniger* strains 54/3 and 75/1-1

An EI mass spectrum determined for the yellow crystalline material from the extract of strain 54/3 (Figure 4.20) matched a previously published EI mass spectrum for geldanamycin (DeBoer *et al.*, 1970; Figure 4.21). Furthermore the UV spectrum (Figure 4.15) for the major component of this extract was also identical to that of geldanamycin (Figure 4.22) published by DeBoer *et al.* (1970). Other known chemical properties for the yellow material were consistent with geldanamycin, including the formation of yellow needle-shaped crystals.

4.3.4.2.4 Evidence for presence of nigericin in extracts of *S. violaceusniger* strains and of isomerisation of nigericin in storage

In order to reduce retention times, modified HPLC conditions had been adopted for the remaining analyses. These conditions were: 80/20 methanol/water to 100 methanol at 15 mins then to 80/20 methanol/hexane at 25 min. Under these conditions the group of minor peaks associated with samples 46/1A-1 and 54/3 (as noted above) were shifted to between 2.5 and 13minutes (from between 21 and 22.4

minutes). MS spectra of these peaks were similar, indicating that the compounds responsible were structurally related. Two HPLC peaks, at 7.8 and 12.3 minutes, (Figure 4.23) were particularly prominent among the group and shared the same main ESI MS fragments (m/e 742 and 747) in differing proportions(Figures 4.24 and 4.25) indicating that the compounds responsible were isomeric. It was believed that 742 corresponded to $M+H_2O$ and 747 to M+Na.

An authentic sample of nigericin was obtained (Sigma, Na salt) and this produced a single HPLC peak at 12.3 minutes (Figure 4.26). The ESI MS spectrum consisted of two main ions corresponding to the water adduct (M+ H_20, m/e = 742.5) and sodium adduct (M+ Na, 747.5) in accordance with corresponding HPLC-MS peaks for samples 46/1A-1 and 54/3. Fragmentation patterns were generated for nigericin for further characterisation (Figure 4.27). The MS-MS spectrum generated from the 747.5 ion consisted mainly of one daughter ion (m/e 729.5), probably corresponding to loss of water from the carboxyl group. Further fragmentation patterns were generated to the great-great-granddaughter level starting with the 729.5 ion.

Recently, Davis *et al.*, (1999) have reported that other compounds from the same group as nigericin (polyether ionophores) were prone to decompose to isomers on storage in aqueous solutions.

MS-MS fragmentation patterns for the fluorescent compound from strain 48/2 (Figure 4.19) were essentially similar to those for authentic nigericin (Figure 4.27) but differed in the absence of an ion with m/e 685.5 at the granddaughter level, indicating that the compound responsible for the fluorescent band was an isomer of nigericin.

A fresh sample of culture metabolites from strain 46/2B was prepared by diffusion from a piece of agar media (cut from a two month old YME culture) immersed in methanol for 1 hour. This sample was compared to authentic nigericin under identical LC-MS conditions. Two HPLC peaks were observed (Figure 4.28) The largest of these, with a retention time of 12.34 minutes, corresponded exactly to nigericin, both in retention time and in MS spectra (Figure 4.29) showing the production of nigericin by strain 46/2B. A second (smaller) peak at 7.85 minutes produced MS fragmentation patterns (Figure 4.30) identical to that for the fluorescent TLC band from strain 48/2 (Figure 4.18). This finding in combination with evidence from literature (Davis *et al.*, 1999) on similar compounds shows that the fluorescent band shared by the

S. violaceusniger strains is the breakdown product from nigericin and indirectly confirms production of nigericin by the remainder of those strains.

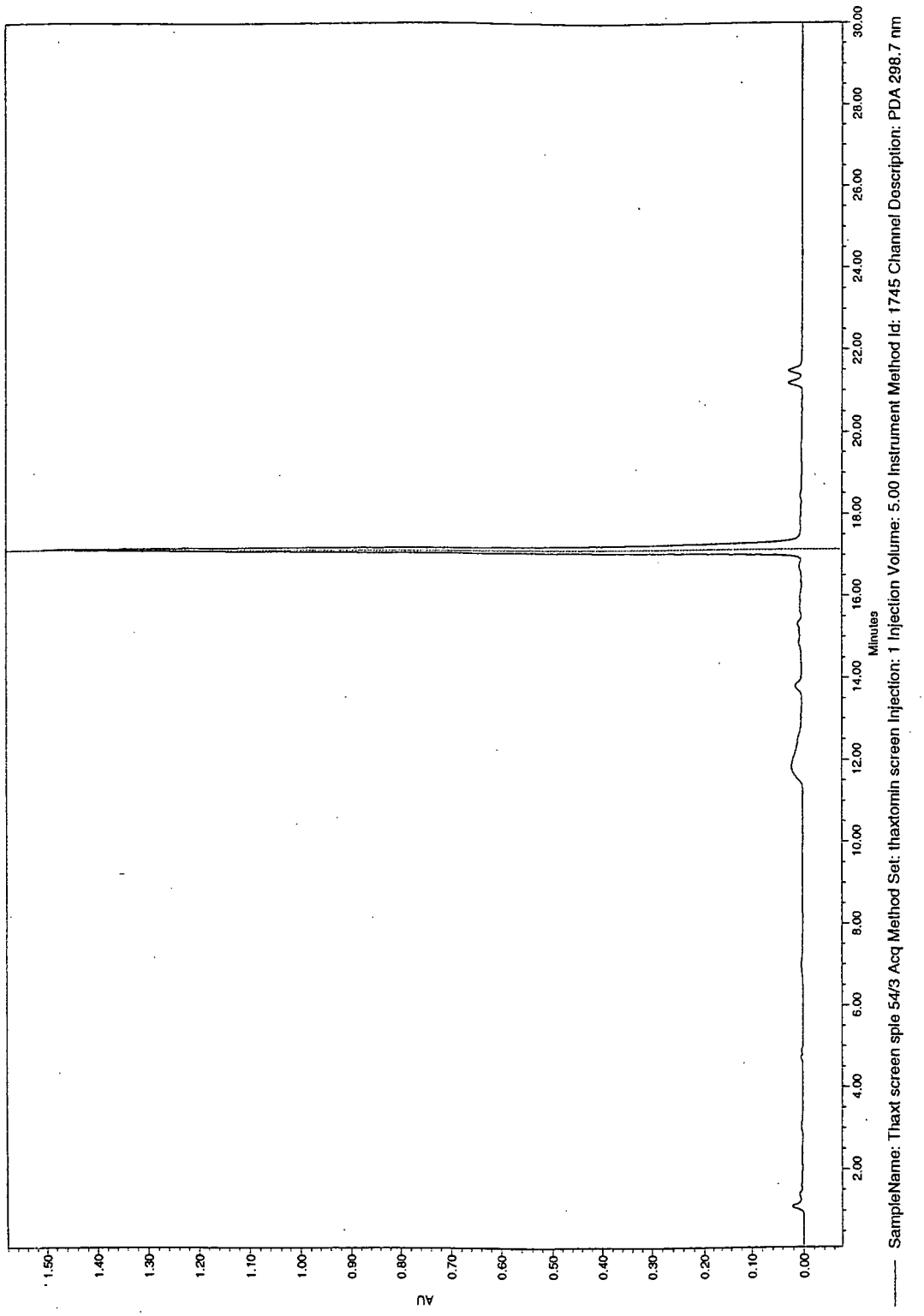


Figure 4.15 HPLC trace using the thaxtomin screening procedure, showing absorbance at 298.7 nm with time, for chloroform extract from OMB culture of *S. violaceusniger* strain 54/3. A large peak is visible at 17.1 minutes.

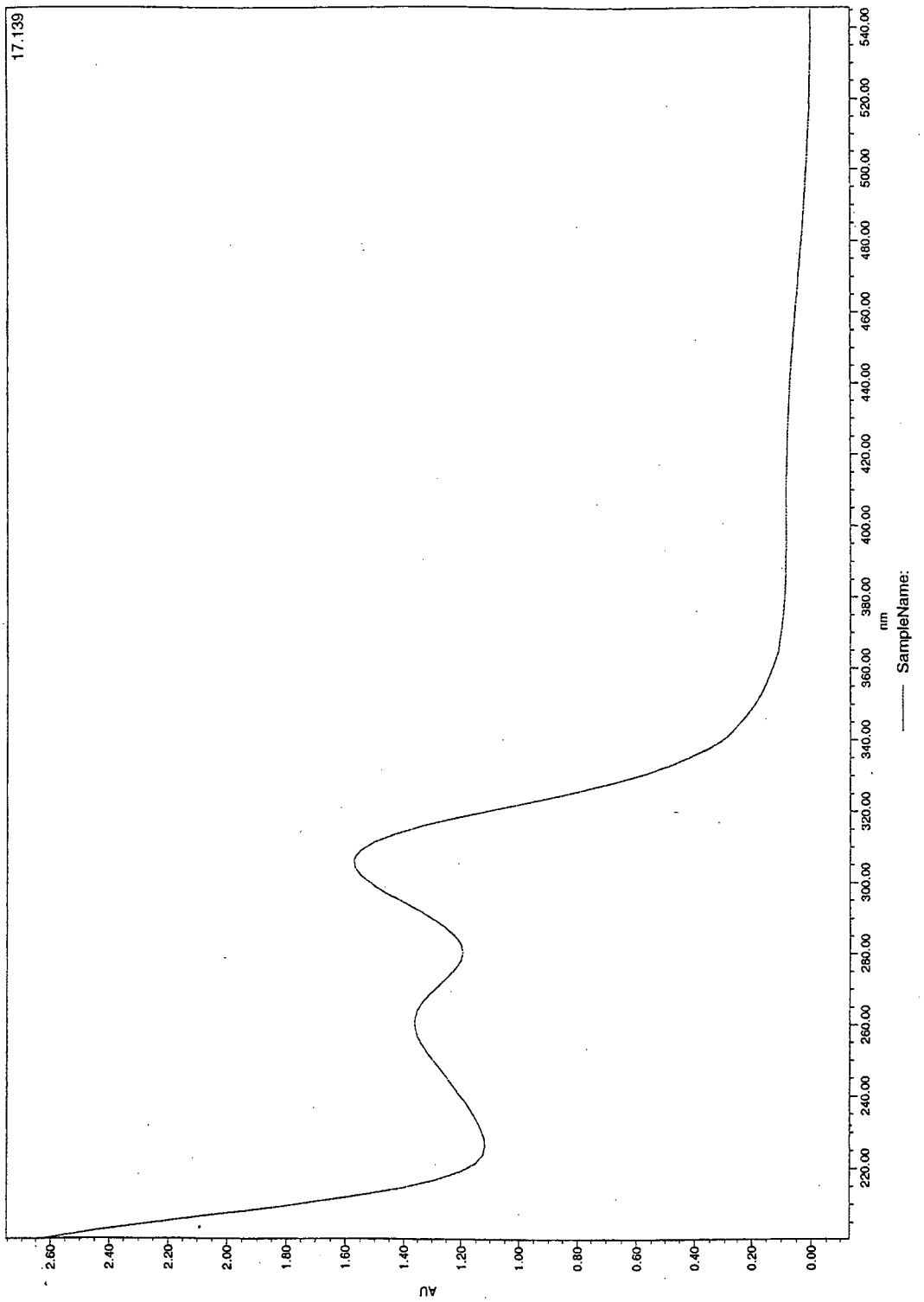


Figure 4.16 UV spectrum for the large peak shown in Figure 4.15.

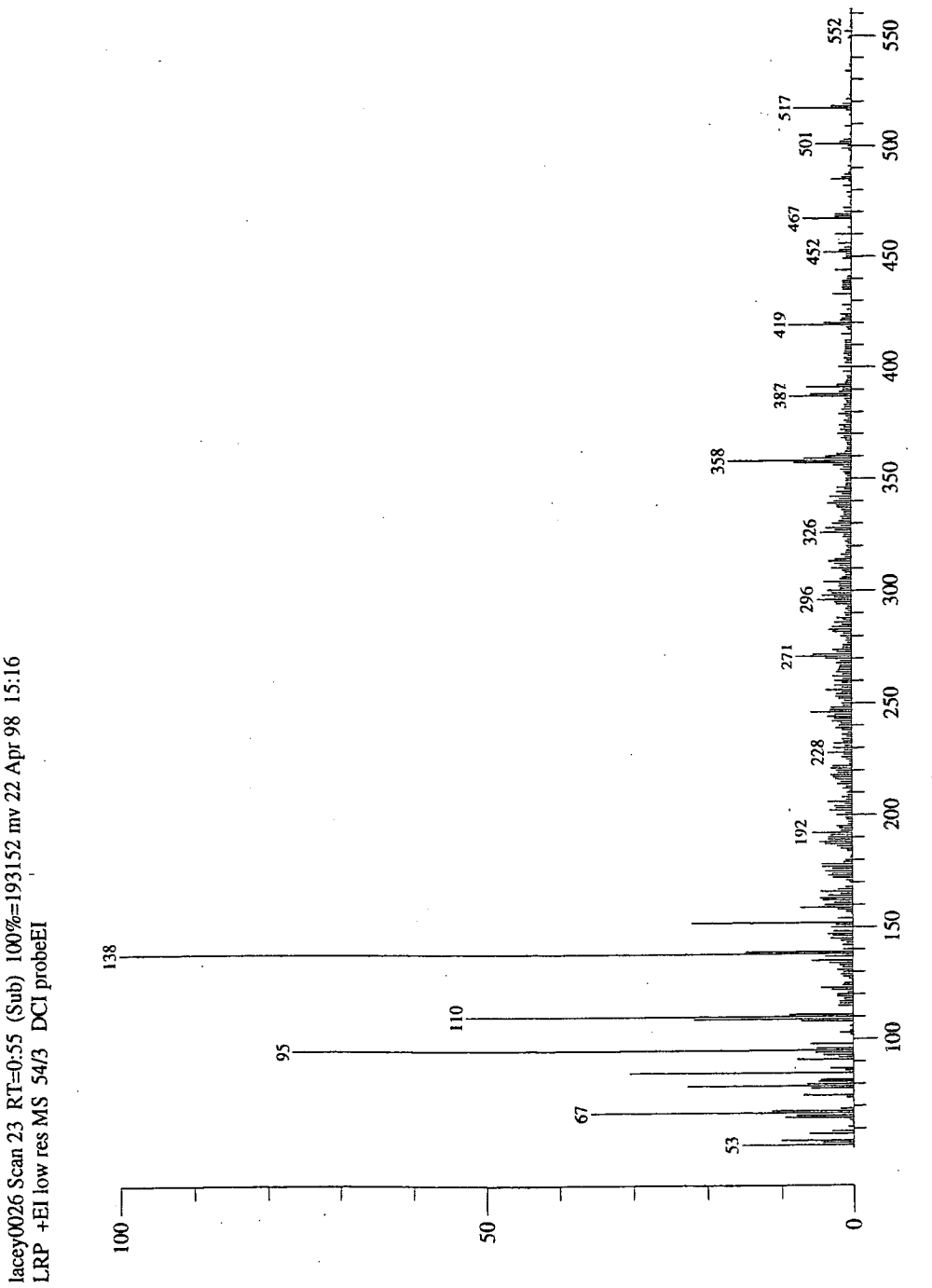


Figure 4.17 EI mass spectrum for chloroform extract from OMB culture of strain 54/3.

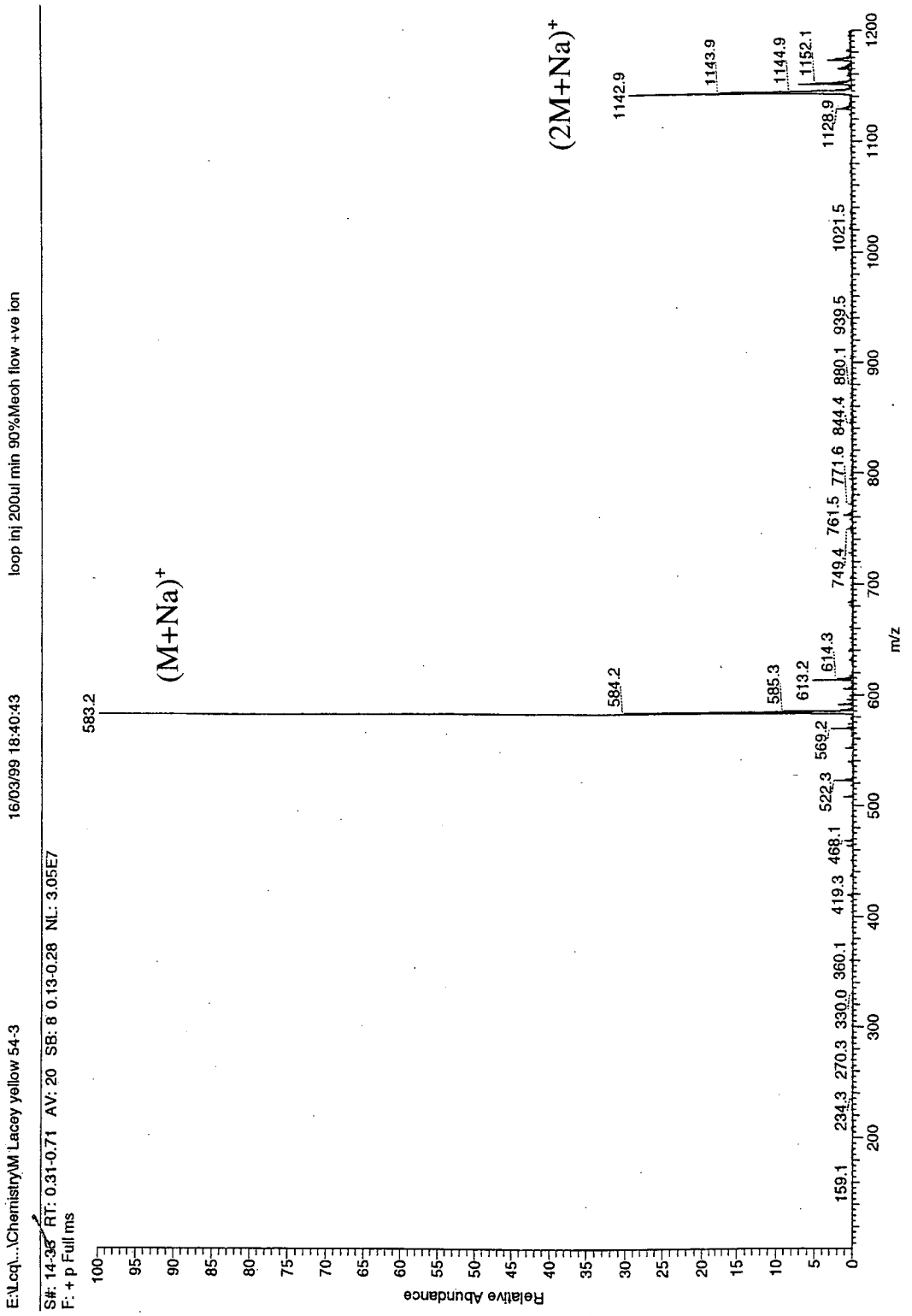


Figure 4.18 ESI mass spectrum for the yellow substance from strain 54/3, showing prominent fragments at m/z 583 (M+sodium)and 1143 (2M+sodium) indicating a molecular weight of 560.

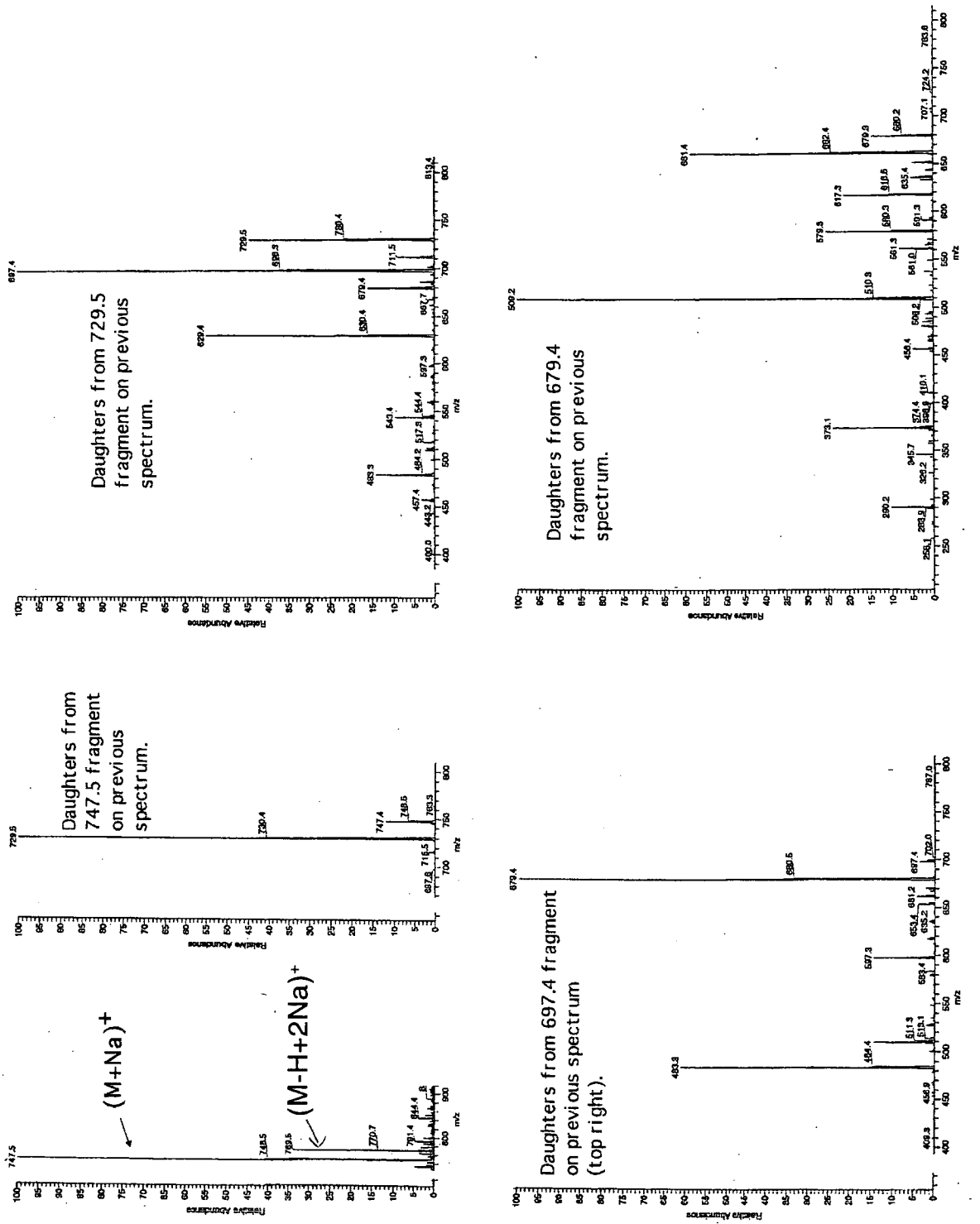


Figure 4.19 ESI MS-MS mass spectral fragmentation patterns for the TLC plate fluorescent band from strain 48/2. First chart shows main ESI fragment at m/z 747. Subsequent charts show consecutive daughter ions generated from major fragments (starting with the 747 fragment shown on the first chart).

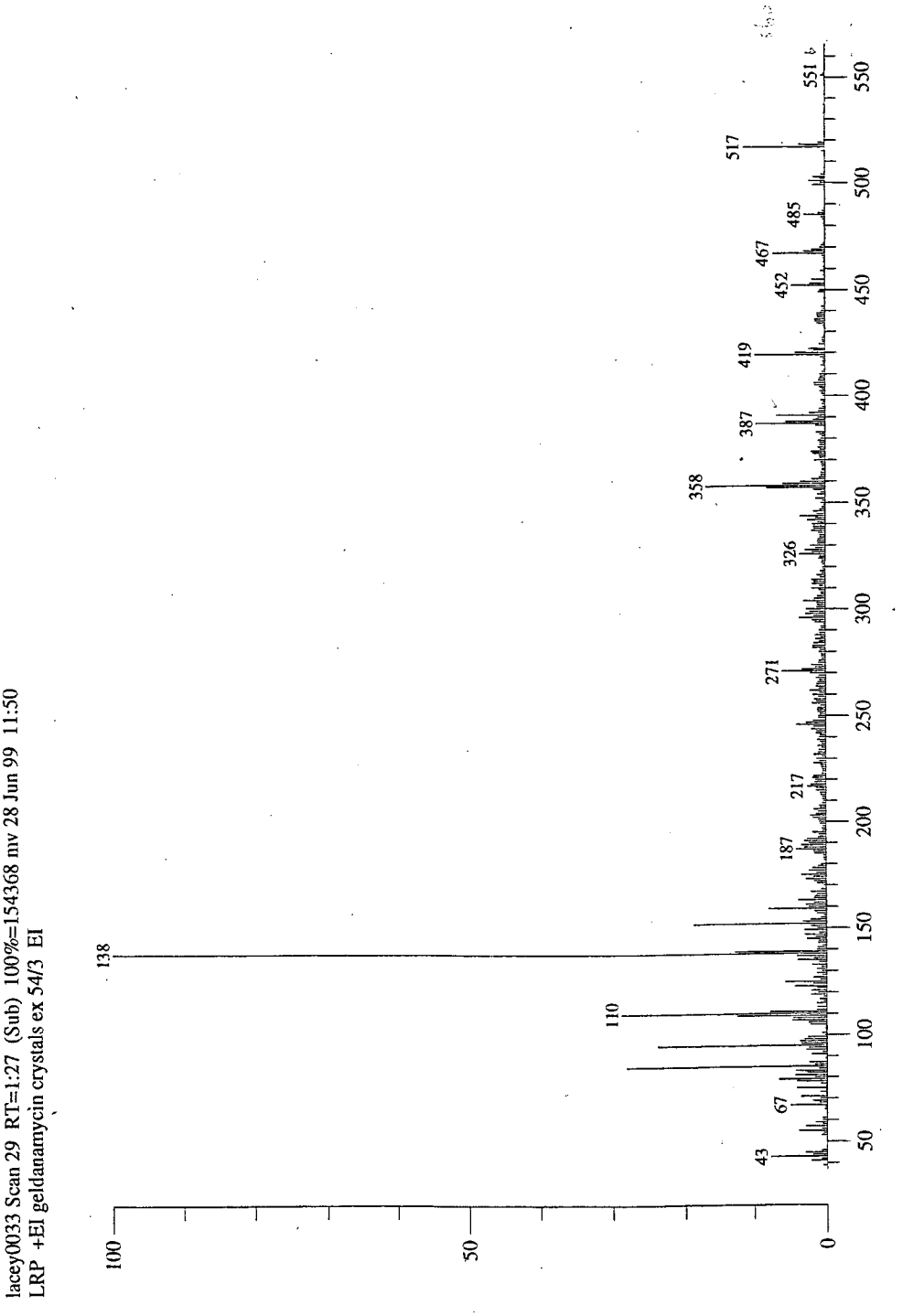


Figure 4.20 EI mass spectrum of yellow crystalline material from chloroform extract of OMB culture of strain 54/3.

M/E	Intensity (%)	M/E	Intensity (%)	M/E	Intensity (%)
41	2.0	203	1.7	389	0.9
44	3.3	204	1.8	390	0.8
45	2.5	206	2.1	391	4.9
46	2.1	216	1.6	392	1.3
79	0.8	217	2.5	419	5.6
85	3.3	218	2.0	420	3.2
91	1.0	219	1.3	421	1.4
94	0.0	220	1.6	436	0.9
95	8.7	222	1.5	438	0.9
96	1.0	245	1.9	441	1.1
108	1.9	246	2.8	452	1.4
109	3.9	248	1.8	453	0.8
110	5.6	271	2.9	468	3.9
111	2.3	272	1.8	469	2.9
138	59.9	282	2.0	470	10.3
139	6.7	304	3.6	471	2.0
140	1.0	326	1.7	483	0.9
152	3.9	328	2.4	484	0.8
159	4.3	330	2.0	485	3.6
166	2.9	331	1.1	486	1.2
167	2.4	342	1.4	487	1.6
172	1.8	344	1.7	499	6.7
173	1.8	346	2.0	500	2.9
178	3.0	358	17.9	501	19.6
187	3.3	359	5.4	502	6.4
188	5.9	360	3.1	503	3.0
189	2.9	361	2.1	513	0.7
190	2.4	370	0.7	515	1.2
191	2.6	374	1.7	517	100.0
192	3.6	381	3.3	518	26.8
193	1.4	382	0.9	519	2.1
194	0.8	386	1.0	560	1.4
195	1.3	387	1.5	562	1.5
202	1.5	388	2.6		

Figure 4.21 EI mass spectral data for geldanamycin (from DeBoer *et al.*, 1970). M/E = fragment mass per unit charge. Intensity (%) is relative to the most intense fragment (m/e 517).

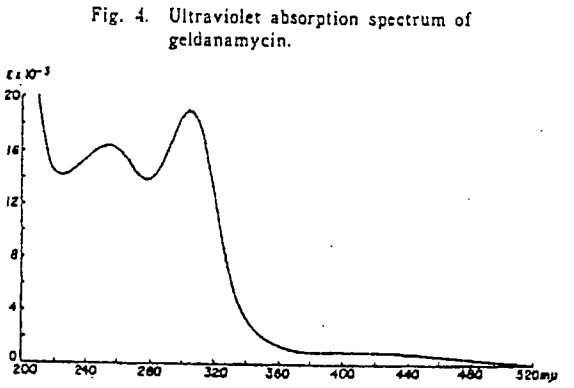


Figure 4.22 UV spectrum for geldanamycin. (Figure from DeBoer *et al.*, 1970).

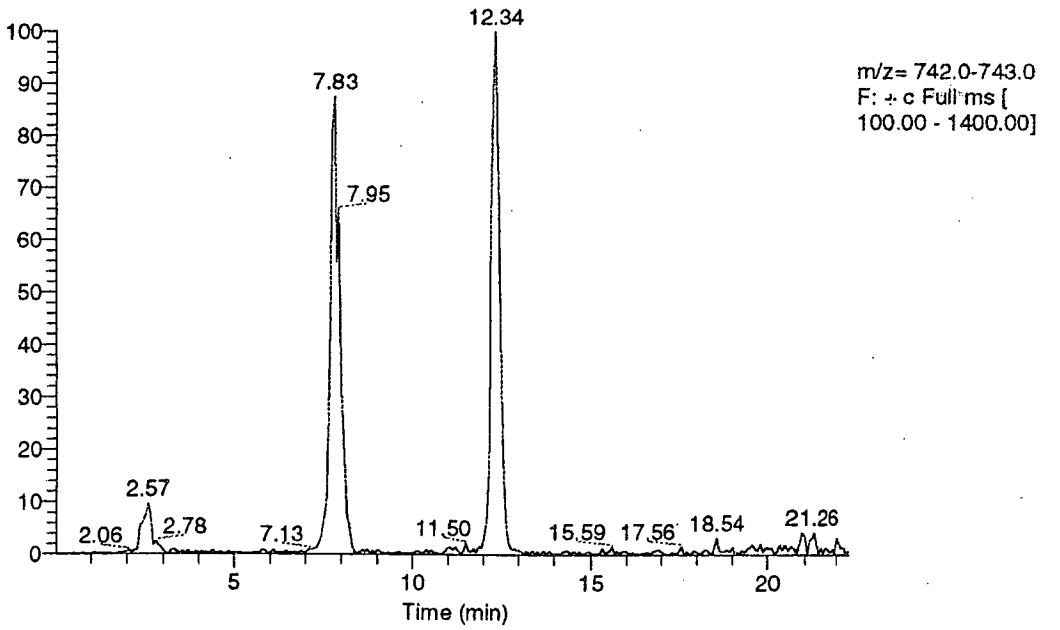


Figure 4.23 HPLC trace for extract from strain 46/2B using modified HPLC conditions. Chart shows two prominent peaks at 7.8 and 12.3 minutes.

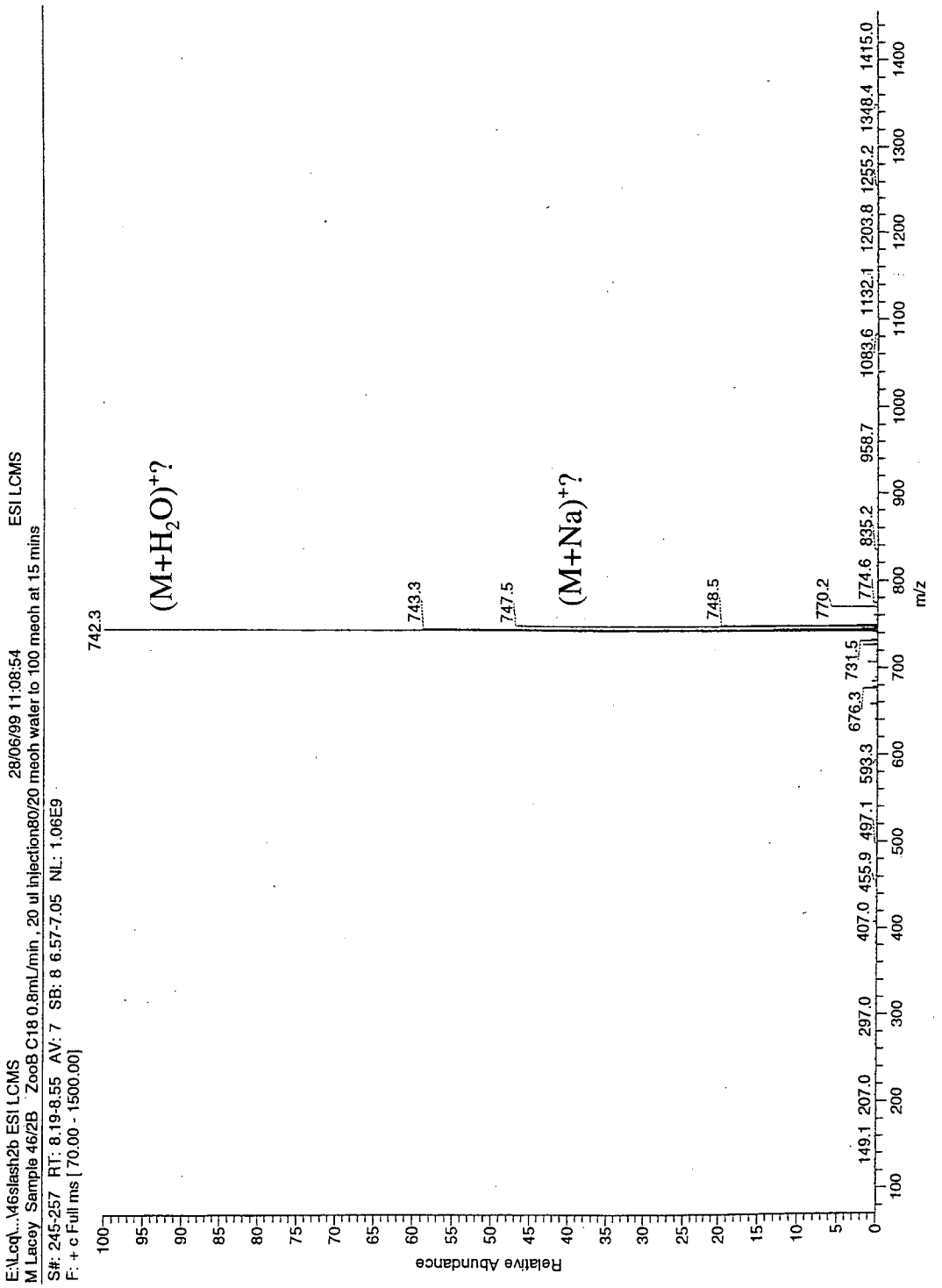


Figure 4.24 ESI mass spectrum for the 7.8 minute peak shown in Figure 4.22.

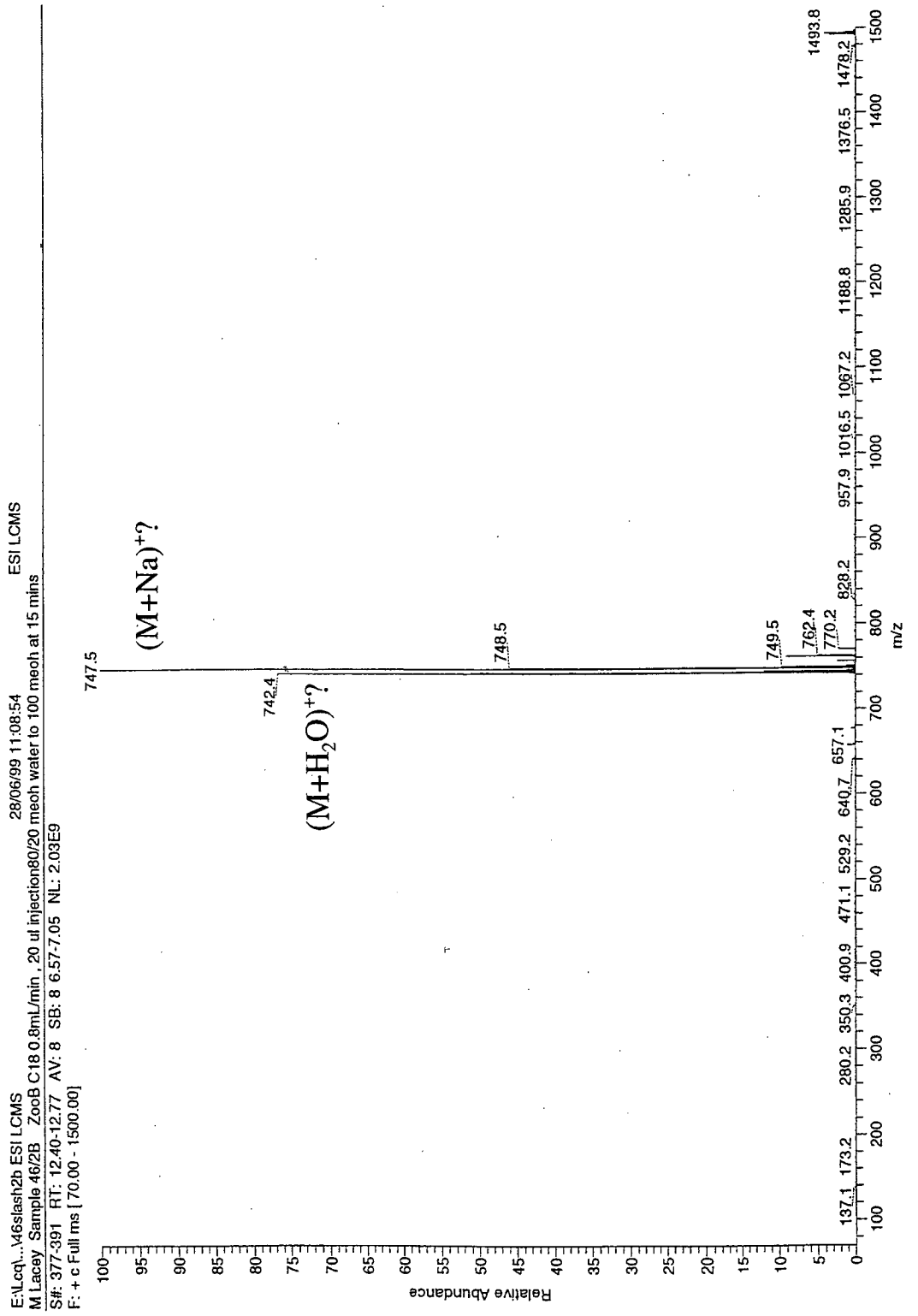


Figure 4.25 ESI mass spectrum for the 12.3 minute peak shown in Figure 4.22.

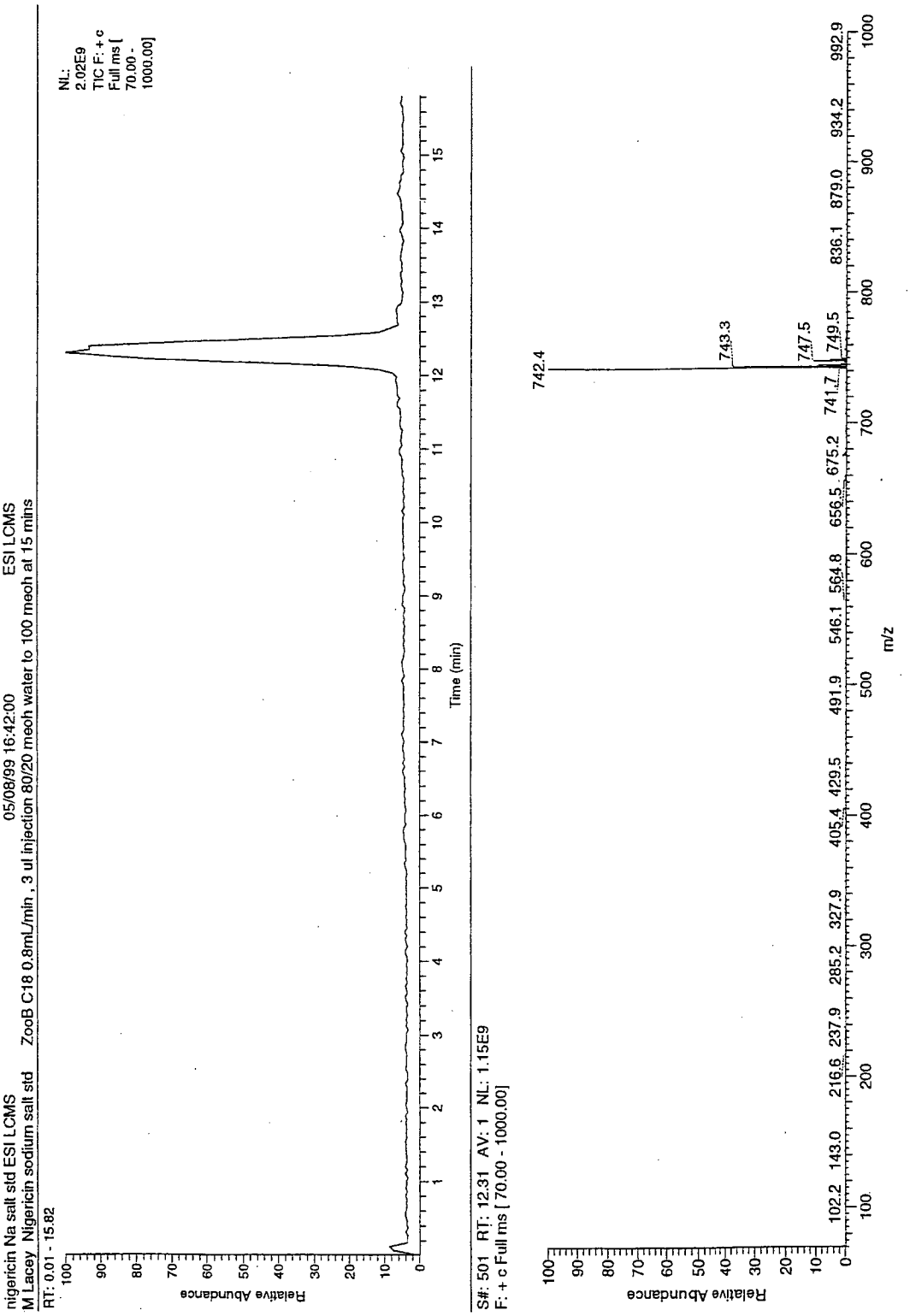


Figure 4.26 LC-MS data for nigericin. First chart shows HPLC trace for authentic nigericin, giving a single peak at 12.3 minutes. Second chart shows the ESI mass spectrum for nigericin, with major fragments at m/z 742.5 and 747.5.

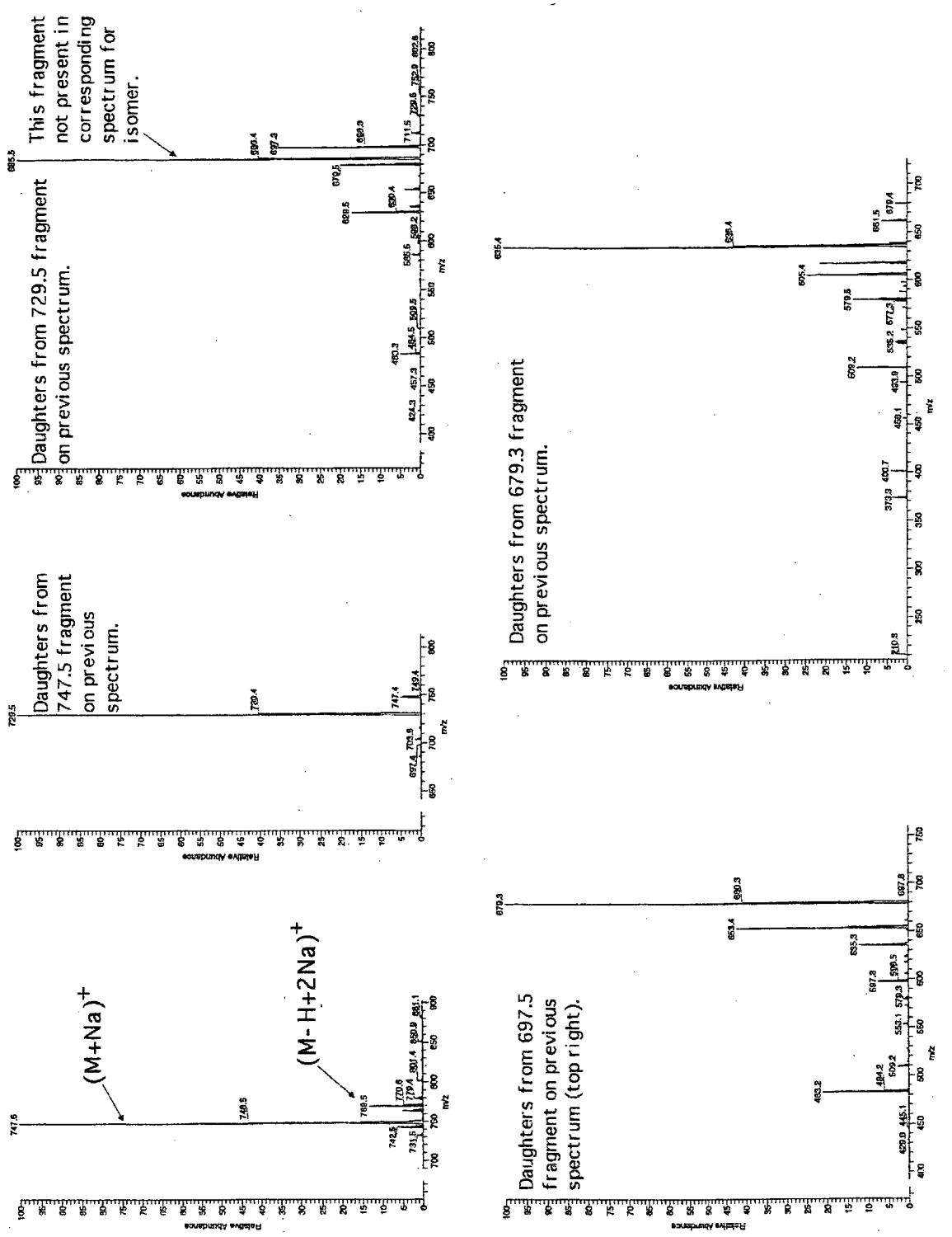


Figure 4.27 ESI MS-MS fragmentation patterns for nigericin, starting with the 747.5 fragment shown in Figure 4.25.

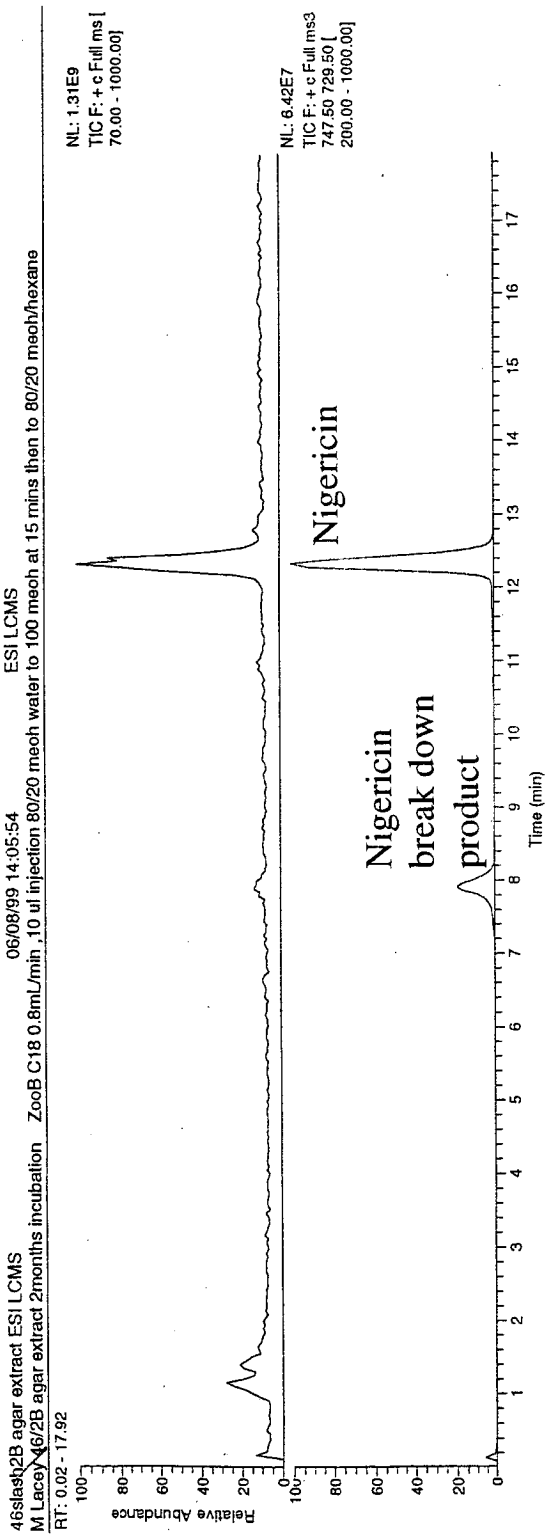


Figure 4.28 HPLC trace for a fresh sample of methanol soluble metabolites from strain 46/2B, showing peaks at 7.8 and 12.3 minutes.

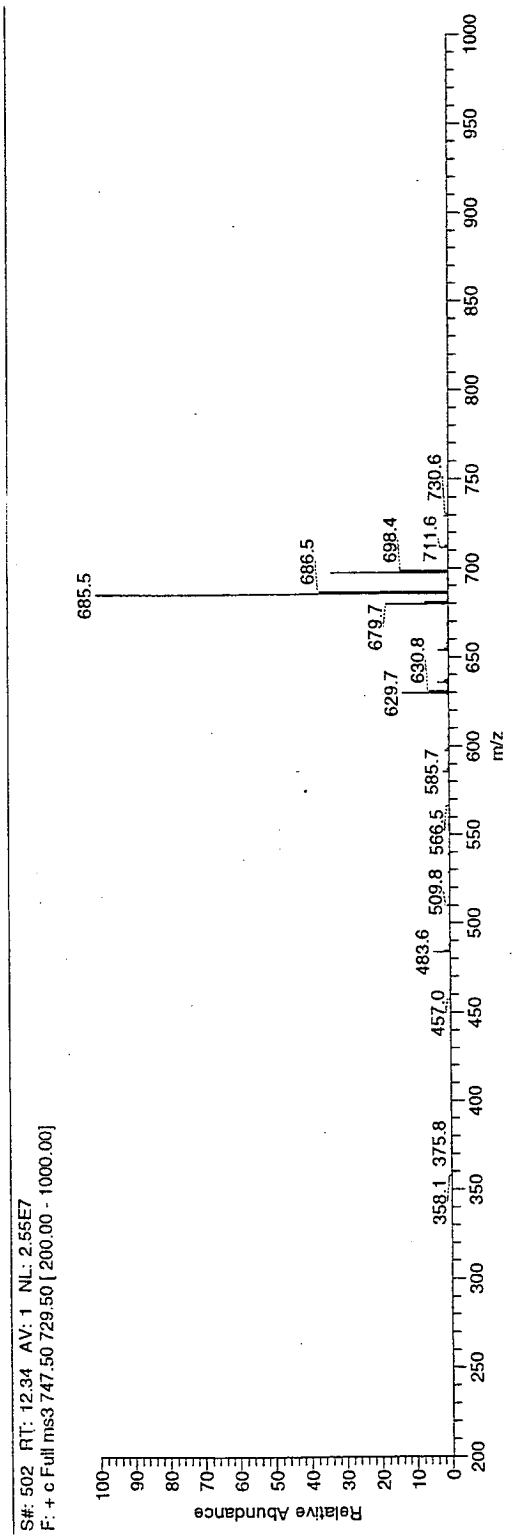


Figure 4.29 ESI MS-MS spectrum for the 12.3 minute peak in Figure 4.27, showing granddaughter ions characteristic of nigericin (Figure 4.26).

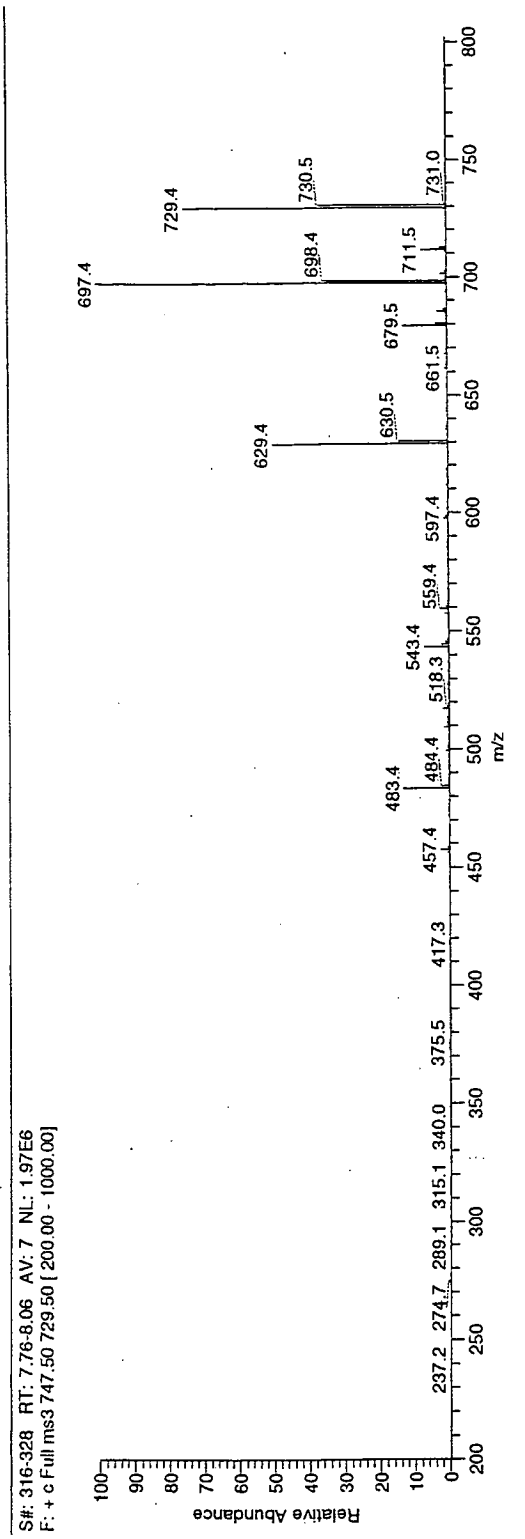


Figure 4.30 ESI MS-MS spectrum for the 7.8 minute peak in Figure 4.27, showing granddaughter ions identical to those shown in figure 4.18.

4.3.5 Co-plating assay and selection of antagonists for glasshouse experiments

Ninety-one strains were screened against strains #27, 46/1A-1, 48/2 and 75/1-1. Assay results are shown in Table 4.6

Two strains showing antagonist potential were selected using the following criteria. Strains: were observed to be not 'pathogenic' as assessed by the potato disk assay; some antagonism to all four of the pathogen strains in the co-plating assays; presence of sporulation of the test strain with inhibition of sporulation of pathogen strains in the co-plating assays; and minimum pH for growth of 5.5 or less.

Table 4.6 Results of co-plating assay

Test Strain	Mean width of inhibition zone[1], (mm).				Sporulation of test strain.[2]				Inhibition of sporulation of lawn strain.[3]			
	Lawn Strain:[4]				Lawn Strain				Lawn Strain			
	A	B	C	D	A	B	C	D	A	B	C	D
1/1	5	2	3	0.5	+	+	+	+	+	+	+	-
1/2-3	5	1	0	0	+	+	+	+	-	-	+	+
1/2-4	2	3	2	0	+	+	+	+	-	+	+	-
2/1-1	0	2	1	5	n.d.[5]	-	-	-	-	+	+	-
2/1-3	2	n.d.	1	2	+	+	-	-	-	n.d.	+	-
2/1-4	0	2	0.5	0	n.d.	+	+	+	-	+	-	-
2/1-5	0	1	0	0	n.d.	-	-	-	-	-	-	-
2/2-2	0	2	2	1	-	-	-	+	-	-	-	+
2/2-3	2	1	0.5	0.5	+	+	+	+	+	+	+	-
2/2-4	0	1	0	1	-	+	+	+	-	-	-	-
2/2-5B	1	2	1	1	+	+	+	+	-	+	+	-
5/1	3	n.d.	2	5	+	+	+	+	-	n.d.	+	-
6/1B	1	2	1	1	+	-	-	+	-	-	-	-
8/2	4	0.5	0.5	0	+	-	-	+	-	+	+	+
9/1	6	0.5	0	0	+	+	-	+	-	+	+	+
12/2A	6	0	0.5	0.5	+	-	-	+	+	+	+	-
12/2B	4	0.5	0	0	+	+	-	+	-	+	+	-
13/1B	6	1	0	0.5	n.d.	+	-	+	-	+	+	+
14/1A	3	0.5	0.5	0	+	+	-	+	-	+	+	-
15/1B	5	0.5	5	0	+	+	+	+	-	+	+	+
15/3A	2	0.5	1	0.5	+	+	+	+	-	+	+	-
16/2-2	4	1	0.5	3	-	+	-	+	-	+	+	+
16/2	5	0	0.5	2	+	-	-	+	+	+	+	-
18/2B	4	0.5	0.5	0	+	+	-	+	-	+	+	-
19/1A-1	2	2	1	2	+	-	-	+	-	-	+	-
19/1A-2	1	14	14	0	-	+	+	+	-	+	+	-
19/1B	6	0	0.5	1	+	+	-	+	-	-	+	-
20/1A	13	1	1	0.5	+	-	-	-	+	+	+	-
20/1B	2	0.5	0.5	0.5	+	+	+	+	-	+	+	-
25/2	4	6	8	0.5	+	+	+	+	-	+	+	-
41/2	0	0.5	0	0	-	-	+	+	-	+	+	n.d.
42/1-2	0	0	0	0	n.d.	-	-	-	-	-	-	-
42/2-1	0	1	1	4	-	-	-	-	-	+	-	-
43/1-1	1	1	1	2	+	-	-	-	-	-	+	-
43/1-2	0	0	0	1	n.d.	n.d.	+	+	-	-	-	+
43/1-3	0	1	0	0	n.d.	-	n.d.	-	-	+	-	-
44/1A-1	9	1	0	0	+	-	-	-	+	-	-	-
46/1A-1	0	0	1	0	n.d.	+	+	+	-	n.d.	-	-
46/1B-1	1	2	0.5	1	+	+	+	+	-	-	-	-
46/2B	0	0	0	1	-	+	+	+	-	-	-	-
48/2	0	0	0	1	-	-	+	+	-	-	-	+
49/1	0.5	0	0	1	-	+	+	+	-	-	-	-
51/1	0	0	0	0	n.d.	+	+	-	-	-	-	-
51/2-1	0	2	n.d.	0.5	n.d.	+	+	+	-	n.d.	-	n.d.
52/2	0	1	2	2	-	-	-	-	-	-	-	-
53/2	0	0	0	0	-	-	-	-	-	-	-	-
54/2	0	0.5	1	0	n.d.	+	+	+	-	-	-	-
54/3	2	1	0	0	+	+	+	+	-	-	-	-

Continued next page

Table 4.6 continued

Test Strain	Mean width of inhibition zone[1], (mm).				Sporulation of test strain.[2]				Inhibition of sporulation of lawn strain.[3]			
	Lawn Strain:[4]				Lawn Strain				Lawn Strain			
	A	B	C	D	A	B	C	D	A	B	C	D
55/2	0	0	0	0	-	-	-	-	-	-	-	-
56/2	0	0.5	1	0	n.d.	+	+	-	-	+	+	-
57/1-2	3	0	0	0	+	-	-	-	+	-	+	-
57/1	1	1	1	1	+	+	-	+	-	+	+	-
59/1	5	3	2	0.5	+	+	+	+	-	-	+	n.d.
62/1-2	0	0	0	0	n.d.	-	-	-	-	-	-	-
62/1	0.5	0	0	1	+	+	+	+	-	-	-	-
63/1	1	0	0	0	+	+	+	+	-	-	-	+
64/1	0	0	0	1	+	-	-	-	-	-	-	-
68/1	0	0	0	0	n.d.	n.d.	+	-	-	-	-	-
72/1-1	3	2	1	2	+	-	+	-	-	+	+	+
72/1-3	0.5	3	0.5	0	+	+	+	+	-	-	-	-
73/1-1	1	2	1	2	-	+	+	+	-	-	-	-
73/1-2	0	9	8	3	+	+	+	+	-	+	+	-
73/1-4	1	2	1	1	+	+	+	+	-	+	+	-
73/1-5	0	0	0	0	n.d.	-	-	+	-	-	-	-
75/1-1	2	1	0	0	+	+	+	+	-	-	+	-
76/1-2	1	2	0.5	1	+	+	+	+	-	+	-	-
77/1	0	0	0	0	n.d.	n.d.	n.d.	-	-	+	-	-
77/3	1	1	2	n.d.	-	-	-	n.d.	-	+	+	+
79/2-1	0	0	0	0	-	-	-	n.d.	-	-	-	-
79/2-3	0	0	0	0	n.d.	-	-	-	-	-	-	-
80/2-1	0	0.5	0	0	n.d.	-	-	-	-	+	+	-
85/1	0	1	0	1	n.d.	-	-	-	-	-	-	-
86/1	3	2	3	0	+	-	-	-	+	-	-	n.d.
88/1	0	n.d.	0	0	n.d.	n.d.	-	-	-	n.d.	-	-
93/2	0	5	5	1	-	+	+	+	-	-	-	-
94/1-1	4	0.5	0.5	0	+	+	-	+	-	+	+	+
94/1-2	0	0	0	0	n.d.	n.d.	+	-	-	-	-	-
95/1	2	2	1	2	+	+	+	+	-	+	+	n.d.
95/2-1	0	n.d.	0	0	n.d.	+	+	-	-	n.d.	-	-
95/2-2	0	0	1	0	n.d.	-	-	-	-	n.d.	-	-
98/1	2	2	1	2	-	-	-	+	-	-	-	n.d.
105/1-1	0	1	0	0	+	+	-	+	-	-	-	-
105/1-2	0.5	2	2	1	+	+	-	+	-	-	-	-
105/2	0	0	1	0	n.d.	+	-	-	-	-	-	n.d.
106/1	0	2	2	2	+	+	+	+	-	n.d.	-	-
108/2	1	2	1	1	+	+	+	+	-	+	+	-
95/13/1A	5	0	0	0	+	+	-	+	-	-	+	-
#23	5	1	0.5	0.5	+	+	+	+	-	+	+	-
#25	0	0	0	0	-	-	-	-	-	-	-	-
#27	0	4	9	2	n.d.	-	-	-	-	+	+	n.d.
#32	0	0	0	0	n.d.	-	-	-	-	+	+	-

[1] Mean zone width measured from edge of test strain spot. Each record is the mean of two replicates

[2] (+) sporulation in at least one replicate;(-) sparse or nil.

[3] (+) inhibition of sporulation in at least one replicate;(-) no inhibition.

[4] Lawn strains were: A) #27 ; B) 46/1A-1 ; C) 48/2 ; D)75/1-1 .

[5] n.d. = not determined

4.4 Discussion

4.4.1 Identity of isolated strains

The majority of 94 streptomycete isolates from scab lesions of potatoes collected from across the Tasmanian potato growing region were sorted into four taxonomic groups based on morphological and physiological characteristics. Groups were: Group 3,*Streptomyces scabies* (25 strains); Group 2, *S. violaceusniger* (11); Group 4, *Streptosporangium* spp. (3); and a group of strains (Group 1) most closely resembling *Streptomyces halstedii* (29). Some of the remaining 26 ungrouped strains resembled *S. scabies*.

4.4.1.1 S. scabies

It is considered likely that the *S. scabies* strains described in this study could represent two distinct forms of the species, with the majority of Group 3 strains complying to one form and *S. scabies*-like strains found among ungrouped strains representing the other.

Members of Group 3 fitted the description of *S. scabies* (Lambert and Loria 1989a), based on characteristics that have been determined. Strains clearly fitted the description in producing grey (predominantly grey brown) spores borne in spiral chains, in production of melanin and in the uses of all nine ISP sugars. This group also included an Australian *S. scabies* reference strain isolated in Victoria (strain #32). Spore surface ornamentation, not included in Thaxter's original description (Thaxter, 1891) but in recent descriptions (Lambert and Loria 1989a; Elesaway and Szabó, 1979), was not determined due to difficulty in preparing specimens for electron microscope examination. Nevertheless the characteristics that have been determined were sufficiently diagnostic for this species. Two strains in this group (#32 and #23) have previously been found to produce smooth spores (Ransom and Gilliam,1991) while strain #32 has previously been shown by R. King to produce thaxtomin A.

Group 3 strains differed in a number of aspects from the descriptions of *S. scabies* (Lambert and Loria 1989a). All grew at pH 4.5 or lower, with 20% growing at pH 4.0. This compares with a published minimum of 5.0 for *S. scabies* (Lambert and Loria, 1989a). In other published accounts, *S. scabies* and *S. scabies*-like strains isolated in

Canada (Faucher *et al.*, 1993; Goyer *et al.* 1996) and Finland (Lindholm *et al.*, 1997) did not grow at pH 4.5. These Group 3 strains also differed from type strains in degrading xanthine and growing in the presence of 0.5 µg/ml crystal violet, 0.1% phenol, 10 IU penicillin-G or 100 µl/ml oleandomycin. With the exception of two strains producing grey spores in loose spirals all strains in this group produced grey-brown spores in tight spirals. A tan or grey-brown colouration of spores has been described for *S. scabies* strains isolated elsewhere including in Canada (Faucher *et al.*, 1992) and Finland (Lindholm *et al.*, 1997).

Among ungrouped strains there were 13 melanin producers which predominantly bore grey spores in loose spiral chains and all-but-two did not degrade xanthine. In these respects this subgroup of strains may comply more closely to the description of *S. scabies* than the Group 3 strains. Of the six *S. scabies*-like strains showing a positive reaction in the potato disk assay, five were from this group. The two group 3 strains with grey spores also had spores borne in loose spirals indicating that they may fit better with the ungrouped *S. scabies*-like strains.

This apparent diversity in *S. scabies* strains is in keeping with findings of other researchers (Healy and Lambert, 1991; Paradis *et al.*, 1994; Ndowora *et al.*, 1996).

4.4.1.2 S. violaceusniger

Eleven strains clearly fitted the description of *S. violaceusniger*. Like *S. scabies* these produced grey spores in tight spiral chains and used all of the ISP sugars. They differed from *S. scabies* in not producing melanin. A pale orange diffusible substance was produced by *S. violaceusniger* strains. In most cases this discolouration was only discernible by comparing inoculated and uninoculated plates of the same medium, meaning that cultures might otherwise have been scored as not producing a diffusible pigment. *S. violaceusniger* strains clearly differed form all other strains in that the spore mass generally disintegrated to form a black mucilaginous material in older cultures. There is evidence that all of these strains produced nigericin.

The *S. violaceusniger* strains could be subdivided into two groups based on physiological characteristics. These groups also differed in apparent pathogenicity. The majority (nine) of the strains did not

degrade xanthine or starch. The two strains that did degrade xanthine and starch also produced geldanamycin, and it is these two strains (54/3 and 75/1-1) which showed the strongest signs of pathogenicity in radish seedling and minituber assays.

S. violaceusniger and *S. hygroscopicus* are often regarded as synonymous and have been grouped together under the name *S. violaceusniger* by (Williams *et al.*, 1983a). Following a study of the DNA relatedness of strains within the *S. violaceusniger* cluster of (Williams *et al.*, 1983a) Labeda and Lyons (1991) have proposed that *S. hygroscopicus* and *S violaceusniger* should be treated as separate species. Under their emended description *S. hygroscopicus* differs from *S. violaceusniger* in not utilising sucrose or raffinose. With this emended description the strains identified in this study remain as *S. violaceusniger*. However it is likely that many strains identified elsewhere as *S. hygroscopicus* might be more correctly ascribed to *S. violaceusniger* and vice versa. It is possible that the geldanamycin producing strains found in this study may be the same as *S. hygroscopicus* var *geldanus*.

4.4.1.3 S. halstedii-like strains

Group 1 strains represented a distinct group of grey-spored streptomycetes, differing from *S. scabies* and *S violaceusniger* in their growth characteristics. The spore mass of Group 1 strains had a more powdery texture than the aforementioned species and substrate mycelia usually had some yellow colouring. Strains most closely fitted the probabilistic description (Williams *et al.*, 1983a) of *S. halstedii*. It is considered likely that members of this group are mainly saprophytic, as evinced by absence of any strong signs of pathogenicity in the potato disk assay. Three strains which were also assayed on radish seedlings and minitubers showed no evidence of pathogenicity.

4.4.1.4 Streptosporangium spp.

Three *Streptosporangium* strains were isolated from potato tubers. Although not commonly isolated, *Streptosporangium* species are believed to represent a significant component of the soil actinomycete population (Nonomura and Ohara, 1969). The isolated strains showed no sign of pathogenicity in the potato disk assay and as a result were not further tested in other pathogenicity assays. These strains had a relatively

high minimum pH requirement for growth (6.5) which would appear to contradict with an apparent preference of *Streptosporangium* for slightly acid, humus rich soils (Nonomura and Ohara, 1969). The *Streptosporangium* strains showed no interaction with lawn isolates in the co-plating assays.

4.4.2 Pathogenicity assays

Pathogenicity of strains was evaluated in four ways. A potato disk assay was used first to screen all strains.

In the potato disk assay, necrosis of the potato tissue in contact with the agar block was expected as the primary indicator of pathogenicity as shown by discolouration and softening of the tissue (Loria *et al.*, 1995). As a secondary indicator, pathogenic strains were expected to colonise and become firmly attached to the tuber disks.

The majority of strains tested showed few strong signs of pathogenicity, the most noticeable exception being *S. violaceusniger* strains which caused distinct necrosis of the potato disks and produced vigorous hyphal growth. In comparison, most *S. scabies* strains produced at best a slight discolouration of the potato disk and some growth on the potato disk, which was designated a weak reaction. Strain #32, a known producer of thaxtomin A, was the most clearly pathogenic of the *S. scabies* strains belonging to Group 3. The lack of a clear response for remaining members of that group indicates that they were, at best, weak pathogens. Five *S. scabies*-like strains among the ungrouped strains were the only others showing a clear pathogenic response.

There appear to be a number of limitations associated with the potato disk assay. Firstly, some variability in the results were noted. The reasons for this variability was not ascertained but may be due to disks being cut from different parts of tubers. Although this variability was observed, replication ensured that a most consistent result could be determined for each strain. However, in the absence of replication this assay may be a less reliable measure of pathogenicity. Choice of potato cultivar did not appear to be a problem. Similar results were obtained for both Kennebec and Bismark tubers in this assay.

A second limitation relates to the suggestions by Loria *et al.*, (1995) that this assay may be a good method to detect thaxtomins in culture media and for identification of pathogenic *Streptomyces* strains.

It is true that this assay may be a good assay for phytotoxins produced by *Streptomyces* strains but it is not necessarily true that those toxins will be thaxtomins unless it is known that thaxtomins are the only phytotoxins produced by those strains. In this study *S. violaceusniger* strains were indicated by the potato disk assay as being the most clearly pathogenic of the strains. However these strains have not been shown to produce thaxtomins but have been shown produce at least one other phytotoxic substance, this being nigericin. At least two strains also produced geldanamycin, another phytotoxin. The phytotoxicity of these compounds on seedlings has previously been demonstrated (Heisey and Putnam, 1986 and 1990). A pathogenic reaction shown in the potato disk assay by an unknown *Streptomyces* strain does not necessarily mean that thaxtomins are produced by that strain. Whether or not those strains which produce phytotoxins other than thaxtomins are pathogens capable of causing common scab symptoms also cannot be determined by this assay.

Following the potato disk assay a smaller selection of strains, including ten *S. violaceusniger*, ten *S. scabies* (Group 3), and three each from *S. halstedii*-like and the ungrouped strains were re-evaluated in further pathogenicity tests. Strains were grown in oatmeal broth then following centrifugation and filtration to remove cellular material, culture supernatants were used in radish seedling and minituber assays. Between radish seedling and minituber assays culture supernatants had been stored for up to one month at -20°C. After an additional five months storage at this temperature, culture supernatants were extracted with chloroform and the extracts were then stored for an additional nine months at -20°C before being assessed for the presence of thaxtomin A and other lipid soluble substances.

In the radish seedling assay, seedling growth was inhibited by supernatants from cultures of *S. scabies* and *S. violaceusniger* strains while seedlings were killed by culture supernatants from *S. violaceusniger* strains 54/3 and 75/1-1. Uninoculated media produced no noticeable growth inhibition. Absence of cellular material meant that in each case the observed inhibition would have been due to presence of phytotoxic substances from the culture supernatants.

S. scabies strains caused seedling growth inhibition and root hair inhibition, consistent with the findings of others (Leiner *et al.*, 1996). Slight swelling of stems of some seedlings inoculated with

supernatants from *S. scabies* strains was also noted, however no histological assessment was conducted to confirm cell hypertrophy expected with thaxtomin action (Leiner *et al.* 1996). Circumstantial evidence is consistent with the involvement of thaxtomin in the observed seedling inhibition, although this evidence may not be conclusive as subsequent mass spectral and TLC examination of 14 month -20°C stored culture extracts revealed no evidence for the presence of thaxtomin A. It is possible that that thaxtomins other than thaxtomin A were responsible for the observed inhibition. Of the *S. scabies* strains, #23 showed the least seedling inhibition in this assay and strain #32 among the greatest inhibition. Previously R. King has demonstrated thaxtomin A production by strain #32 but not strain #23 (R. King, pers. com.), a result independently confirmed by G. Luckman (pers. com.). Production of thaxtomin A by strain #32 in a separate OMB culture was also demonstrated in the current study. These observations may lend support to the possible presence of thaxtomin A or other thaxtomin compounds.

Even in the absence of thaxtomin, inhibition of seedlings by *S. violaceusniger* strains can be explained by their production of nigericin and geldanamycin. For those strains for which nigericin (but not geldanamycin) production could be demonstrated, seedling growth was inhibited but no necrosis was observed. Radical growth and root hair formation was inhibited although root growth of some seedlings partially recovered via root branching (unlike results for *S scabies* strains, where root branching was not observed). Culture supernatants from the two geldanamycin producing strains (54/3 and 75/1-1) caused necrosis of all inoculated seedlings within 24 hours. These effects of *S. violaceusniger* strains on seedlings are consistent with similar findings of Heisey and Putnam (1986 and 1990). They found that nigericin and geldanamycin produced by *S. hygroscopicus* (syn *S. violaceusniger*) were inhibitory to seedlings of cress (*Lepidium sativum* L.) and other plant species. Although both compounds inhibited seed germination and slowed seedling growth only geldanamycin was observed to cause necrosis. These results are congruous to the current findings.

Little or no seedling inhibition was noted for *S. violaceusniger* strains 54/2 and 94/1-2. This may be accounted for by slower growth of these strains in the broth medium. Carbohydrate material remained in these broths at the end of the eight day growth period as indicated by greater opacity of the medium and significantly increased time required

for filtration. These cultures may have not reached the end of the exponential growth phase at which time production of secondary metabolites, including antibiotics, is induced by many *Streptomyces* species (Demain *et al.*, 1983).

Strains tentatively identified as *S. halstedii* showed little or no seedling growth inhibition, providing further evidence that these strains are not pathogenic

Ungrouped strain 2/1-3 showed little seedling inhibition although the potato disk assay had indicated pathogenicity. No clear explanation for this difference could be determined. Strain 2/1-3 is likely to be *S. scabies* as indicated by similarity in banding pattern on TLC of culture extracts.

Evidence of pathogenicity was shown in the radish seedling assay by selected antagonist strain 25/2 but not by strain 2/2-5.

The radish seedling assay has the advantage that it is quick and easy to conduct and would be useful for screening strains for production of toxic substances. As with the potato disk assay, this assay does not necessarily indicate that the strains produce thaxtomin or are capable of causing common scab symptoms. A reaction on radish also does not necessarily indicate that a similar reaction would be found with potato.

In the minituber assay, *S. scabies* strains showed little or no reaction, with inoculated tubers being mostly indistinguishable from the controls. The exception being three strains for which slight darkening of lenticels had been shown. These strains (#32, 1/2-3 and 12/1A) had also been amongst the most inhibitory in the radish seedling assay and had shown some reaction in the potato disk assay. On the basis of the minituber assay results alone it could be concluded that many if not all of the *S. scabies* strains were non-pathogenic. However combined with the results of the other assays there is some evidence of pathogenicity for at least three strains. Lack of scab-like necrosis observed for *S. scabies* strains in the minituber assay may indicate that the thaxtomin concentration (if present) in the media was low, although this may not be consistent with the results of the radish seedling assay in which the same broth medium had been used. It is possible that a lower concentration of thaxtomins may have been required to produce a measurable effect on radish seedlings than on minitubers. Thaxtomin may have been present at sufficient concentration in the radish seedling and minituber assays to

inhibit plant growth without causing visible necrosis. This may have been more evident on radish seedlings due to their otherwise more obvious expansion during growth than on minitubers. Lawrence *et al.*, (1990) observed scab induction on potato minitubers by thaxtomin A at 10^{-5} M and thaxtomin B at 10^{-6} M. Leiner *et al.* (1996) observed effects of thaxtomin A on radish seedling growth at concentrations at or above 10 μM. However concentrations in the range 25 to 50 μM were required for cell death. An alternative explanation for the apparent difference between results of the two assays may be that pathogenicity factors other than thaxtomin may have played a part and had a greater effect on radish seedlings than on potato minitubers.

In contrast to results for *S. scabies* strains, *S. violaceusniger* strains had a very clear effect on minitubers. Necrosis of both green leafy tissue and lenticels occurred within 24 hours of dipping tubers in culture supernatant from *S. violaceusniger* strains. As with the radish seedling assay, strains 75/1-1 and 54/3 had the most severe necrotising effect, causing scab-like necrosis of a significant proportion of tuber surfaces.

Two possible explanations for the non-detection of thaxtomin in chloroform extracts include: 1) volume of broth media may have been insufficient for a measurable quantity to be extracted and ; 2) degradation may have occurred in storage.

Volume of media extracted was between 49 and 77 ml per strain. As thaxtomin A is normally produced in microgram quantities per millilitre of media (Loria *et al.*, 1995; Beauséjour *et al.*, 1999), the volume may not have been sufficient for detection. When strain #32 was grown in a larger volume (500ml) of media thaxtomin A could, however, be detected by TLC. This volume was nevertheless insufficient for detection of thaxtomin from: *S. scabies* strains 12/1A, 1/1; *S. violaceusniger* strain 54/3 or; strain 85/1 indicating that these strains were low thaxtomin A producers or thaxtomin A non-producers.

Degradation of thaxtomin A could have occurred during the sample storage period, it being 14 to 15 months between when the radish seedling/minituber assays were conducted and when assays were first conducted to assess thaxtomin content of the media extracts. Samples were stored at -20°C in attempt to minimise degradation. There is evidence that thaxtomin degradation can occur at this temperature. G. Luckman (pers. com.) has found significant loss of thaxtomin A on

storage of extracts from a broth culture of *S. scabies* strain #32. This degradation had been observed to occur if co-extracted lipids had not been removed prior to storage, as was the case in the experimental procedures described here. There are no published accounts quantifying the degradation of thaxtomins. An experimental protocol could be designed to quantify these effects. For example, a known starting concentration of thaxtomin could be stored at a range of temperatures (including -20°C) as part of a crude extract and in broth media, and then assayed at intervals. Such assays could look for thaxtomins and likely degradation products.

In the present study, culture conditions should have been sufficient for at least some thaxtomin production. Babcock *et al.* (1993) found that the optimal temperature for thaxtomin production by *S. scabies* in oatmeal medium was 28°C. In the presently described work, culture incubation had been at a variable temperature between 15°C and 30°C with the average probably in the vicinity of 20°C. Incubation time of 8 days would have been sufficient as relevant cultures had reached the end of the exponential growth phase. Measurable quantities of thaxtomin A were also produced by *S. scabies* strain #32 grown at 20°C. Glucose inhibition of thaxtomin production (Babcock *et al.*, 1993) is unlikely to have been a problem as no glucose had been added to the medium

Pathogenicity was not conclusively shown on potato plants growing in sand for any of eight strains tested. A small number of atypical lesions were observed for each of two *S. scabies* strains (including strain #32, a known positive control) and two *S. violaceusniger* strains, however because lesions were mild, few in number and atypical and the inoculated strains could not be reisolated from those lesions (thus proving Koch's postulates) it was not possible to conclusively confirm the pathogenicity of those strains. Pathogenicity of *S. scabies* strains #32 has been previously demonstrated. Hence it is considered likely that lack of disease in this assay may have been a reflection of sub-optimal conditions for scab development.

It is unlikely that the choice of potato cultivar had been a limiting factor in the pathogenicity assays. Kennebec, a moderately scab resistant (Powelson *et al.*, 1993) potato cultivar was used for pathogenicity testing in minituber, potato disk and pot assays. This cultivar has been successfully used for pathogenicity testing by other researchers (Babcock *et al.*, 1993; Liu *et al.*, 1996; Goyer *et al.*,

1998).The cultivar Bismark was also used in the potato disk assay with similar results to Kennebec.

Other potato cultivars that have been used elsewhere in pathogenicity testing are varied and include 'Chippewa' (Loria and Kempter 1986; Loria *et al.*,1995), 'Green Mountain' (Lawrence *et al.*, 1990; Faucher *et al.*, 1992 & 1993) 'Conestoga' (Faucher *et al.*, 1992; Goyer and Beaulieu, 1997), 'Pontiac' (Babcock *et al.*, 1993) 'Yukon Gold' (Goyer and Beaulieu, 1997) and 'Matilda' (Lindholm *et al.*, 1997).

On balance it is considered likely that at least some of the Tasmanian isolated *S. scabies* strains evaluated in this study may be pathogens, although the evidence is not strong. The strongest evidence was shown in the radish seedling assay, where consistent inhibition was found for most *S. scabies* strains. The pattern of inhibition was consistent with that expected for *S. scabies* and differed from that shown by *S. violaceusniger* strains. In this assay, strain #32 a known pathogen that is also known to produce thaxtomin A, gave identical results to other *S. scabies* strains. The three *S. scabies* strains (including #32) showing some evidence of necrosis in the minituber assay were amongst those showing greatest inhibition in the radish seedling assay. It is possible that pathogenicity factors other than thaxtomin are important in pathogenicity of *S. scabies*. Bukhalid and Loria (1997) found that when the *nec1* gene was cloned in an otherwise non pathogenic strain of *S. lividans*, the transformed strain could produce scab-like lesions on immature potato tubers. This transformed *S. lividans* strain did not produce thaxtomin A but did produce an unidentified phytotoxic substance. The involvement of extracellular enzymes, such as esterases, in pathogenicity of *S. scabies* has been proposed (McQueen and Schottel, 1987; Beauséjour *et al.*, 1999) and may warrant further consideration. A low incidence of pathogenicity among *S. scabies* isolates may not be unusual. Keinath and Loria (1989b) in a study of population dynamics found that fewer than 6% of *S. scabies* strains isolated from soil and tuber surfaces were pathogenic.

Observations, described here, of effects of *S. violaceusniger* strains on potato disks and on minitubers represents the first known record of herbicidal effects on potatoes by *S. violaceusniger.* It has been shown that *S. violaceusniger* can grow readily on and cause necrosis of potato tuber tissue. Scab-like necrosis of minitubers which have been dipped in supernatant from *S. violaceusniger* cultures has also been

demonstrated. If such necrosis can be reproduced on tubers grown in soil inoculated with a *S. violaceusniger* strain this would show the capacity of *S. violaceusniger* to cause a novel form of potato scab.

4.4.3 Relationship between apparent pathogenicity and source lesion type

No clear relationship between source lesion type and apparent pathogenicity of strains as determined by the potato disk assay was demonstrated from any of the species groups. Approximately equal numbers of strains in each group were sourced from russet and erumpent lesions. Interestingly, all strains isolated from pitted lesions scored either a nil or weak reaction in the potato disk assay and may suggest that pathogen strains had been missed in these and possibly other isolations.

4.4.4 Selection of antagonists

Two strains with presumed antagonist potential were selected for use in biocontrol studies. The primary criteria for selection was their response to four presumed pathogens in co-plating assays. In addition to inhibition of all pathogen strains, as shown by the presence of an inhibition zone, also evidence of capacity for sporulation in the presence of the pathogen was also taken into account. Additional criteria for selection were that the strains were non-pathogens (based on response to the potato disk assay) and were capable of growth in the same pH range as pathogenic strains.

The two selected strains were 2/2-5 and 25/2. Strain 2/2-5 was not strongly antagonistic but consistently showed some inhibition of all four test strains. Strain 2/2-5 is typical of taxonomic group 1, tentatively identified as *S. halstedii*. The other antagonist (25/2) was more strongly antagonistic to the test strains. It is believed that 25/2 may be a strain of *S. scabies* as shown by similarity in banding pattern observed on thin layer chromatography of broth culture extracts (Figures 4.12 and 4.13). This strain differs from *S. scabies* in producing spores in flexuous chains. Strain 25/2 may also be pathogenic to some extent as shown by inhibition of radish seedling growth (Figure 4.7) and as a result it does not truly fit the original selection criteria.

The usefulness of these two strains as scab antagonists has also cast into doubt by the subsequent discovery that three of the four

presumed pathogens used for screening in co-plating assays are strains of *S. violaceusniger* and not *S. scabies*. In effect the antagonists have been primarily selected for antagonism to *S. violaceusniger*. The identity of the fourth pathogen (strain #27), sourced from the DPIWE culture collection (Ransom and Gilliam, 1991), was not determined although there was evidence that this strain was not typical of *S. scabies*.

Regardless of the outcome, the antagonist strains were selected following a predetermined procedure, and were subsequently used in experimental studies. It is for this reason that aspects related to their selection has been documented and discussed here.

It can be assumed that the surface of potato tubers might be a good source of antagonist strains, as such strains would need to be capable of colonising the surface of potato tubers and competitive exclusion of the pathogen from this ecological niche. Such a strategy has been successfully used by other researchers such as Liu *et al.*(1996). In addition, these researchers have focussed on antagonists sourced from potatoes grown in scab suppressive soil such as that found in a potato research plot a Grand Rapids, Minnesota (McQueen *et al.*, 1985; Lorang *et al.*, 1989; Liu *et al.*, 1996). Such soils can be expected to be a more efficient source of antagonists than soil that is not scab suppressive.

There are no known sites in Tasmania where scab suppressive soils have been either suspected or demonstrated. It follows that if such a site were to exist that it would be a suitable source for locally adapted antagonists. There is no reason to believe that such suppressive soils could not develop in the future in areas that are heavily used for potato production.

Ironically, *S. violaceusniger* may prove to have potential as an antagonist if it is shown to be not scab promoting. Rothrock and Gottlieb (1984) have shown that geldanamycin producing *S. hygroscopicus* var. *geldanus* (syn. *S. violaceusniger*) can cause antagonism to *Rhizoctonia* in soil, although they also observed that *S. hygroscopicus* and geldanamycin were inhibitory to plant growth. The fact that *S. violaceusniger* strains have been isolated from scabbed potatoes in the present study may indeed be evidence that they are acting as scab antagonists. Further experimental work would be needed to resolve the pathogenicity of *S. violaceusniger* strains on potato and to demonstrate any scab antagonist potential.

5 The Soils

5.1 Introduction

Krasnozems are the predominant soil type of the Tasmanian potato cropping region. These soils, also known as ferrosols, are highly regarded for their favourable agronomic properties (Isbell, 1994). Tasmanian krasnozems soils are derived from tertiary basalt deposits and are mainly found in the state's north west (Appendix 3.1). Isbell (1994) described krasnozems as being red to brown, strongly structured acid clay soils with depth ranging from 1 to 7 metres. As kaolin is the predominant clay mineral, with significant amounts of aluminium and iron oxides, these soils have variable charge properties with low cation exchange capacity and usually significant anion exchange capacity. Organic matter is the main source of cation exchange capacity and in some krasnozems can contribute as much as 70% of the effective cation exchange capacity in the surface 10 cm (Gillman, 1976). For sustainable use, these soils are dependent on maintainance of organic matter, maintainance of pH by application of amendments such as lime, minimisation of erosion and replacement of nutrients lost through cropping. (Moody, 1994).

In 1994 the krasnozem soils of north-west Tasmania were surveyed by the DPIWE for soil factors associated with common scab (Wilson, 1996). In this survey, soil and tubers had been sampled from 31 sites over 25 localities. Tubers were rated for scab severity and associated soils subjected to chemical analyses. This survey found no single clearly discernible scab conducive factor amongst those measured. In large part, results were confounded by the variety of agronomic practices represented over the sampling range, meaning that measures of scab between sites may not have been strictly comparable. A concurrent assessment of microbiota found in these soils showed no differences in actinomycete or bacterial counts. Soil chemical and scab severity data from the 1994 DPIWE survey is included in Appendix 3.2.

An additional soil and tuber sampling program was conducted in 1995, using the same sampling methods as in 1994 but without detailed scab assessment and again focussing mainly on krasnozems.

The material collected in the 1995 survey formed the basis for experimental work described here.

The goal of this work was to determine if common scab conduciveness determined under controlled conditions for a selection of Tasmanian potato cropping soils could be correlated with any of 12 chemical properties of those soils.

5.2 Methods and Materials

5.2.1 Scab survey soils (1995)

Soil samples (predominantly krasnozem) which had been collected from the vicinity of potato tubers sampled during the 1995 common scab survey were obtained from DPIWE. These samples had been stored in plastic bags at 4°C for approximately twelve months since collection. Thirty six of the 108 samples were randomly selected for detailed study. A range of chemical and physical properties known or suspected to have some influence on common scab and a measure of scab severity was determined for each sample.

5.2.2 Analysis of samples

Each soil sample was mixed thoroughly to ensure uniformity before subsamples (300g) were collected and air dried. Analysis of twelve chemical and physical properties was carried out on these subsamples by a commercial laboratory (Allison Laboratories) using Australian standard methods. Properties evaluated were: loss on ignition (a measure of organic matter content); pH; electrical conductivity; exchangeable P, K, Ca, Mg; trace elements (Mn, Zn, Cu, B); and Kjeldahl nitrogen. Extraction procedures used in chemical analyses were: pH, conductivity - 1 part soil to 5 parts distilled water; P - 1 part soil to 20 parts 1 N sodium bicarbonate at pH 8 (Olsen); K, Mg, Ca, B - 1 part soil to 5 parts 1 N ammonium acetate at pH 4.8; Mn, Cu, Zn - 1 part soil to 10 parts 0.01 N EDTA at pH 4.

5.2.3 Scab survey soils pot trial

No common scab data was available from the field sites from which the 36 soils described above had been sampled. However, such data if available would have been of limited use given the diversity of agronomic practices at each site in the year of survey. A scab rating was instead determined by growing potatoes in the soils under controlled conditions. Soils were each placed into two or more (depending on the amount of soil available) 15 cm diameter pots. The ability of these soils to suppress disease was also assessed by addition of common scab inoculum to half the number of pots of each soil. Where there were an odd number (2n+1) of pots for a sample, inoculum was applied to the

greater number (n+1) and the remainder (n) were not inoculated. This unequal replication affected 22 of the 36 samples. Spores scraped from the surface of plates of three week old YME cultures of pathogenic *S. scabies* strain #32 were suspended in water and applied evenly to the soil surface of the pots at approximately $2.3x10^9$ spores per pot.

One eye-core of potato cultivar Russet Burbank was sown per pot. Plants were grown under normal glasshouse conditions with minimal watering (Figure 5.1). At harvest, scab severity of tubers was measured.

Figure 5.1 Survey soils pot trial. Soils were sown with potato cultivar Russet Burbank.

5.3 Results

5.3.1 Scab survey soils pot trial

5.3.1.1 Scab severity

Scab severity observed in this pot trial was mild with only slight scabbing of tubers in 16 out of the 36 soils. Scab severity was calculated as percentage of tubers with some scab. Results are shown in the second column of Table 5.1.

There appeared to be little effect of added pathogen inoculum with 13 of the 36 inoculated soils showing scab as opposed to eight of 36 unamended soils. Unequal pot replication between inoculated and uninoculated soils may have accounted for much of the difference as scab was not found in all pots. No scab was observed with 20 of the 36 soils. The appearance of scab lesions did not differ between inoculated and uninoculated pots. Because of the limited replication and low scab severity, the influence of added inoculum on scab severity could not be reliably compared.

5.3.1.2 Correlation of scab severity with soil chemical analyses

Data for inoculated and un-inoculated soils was pooled for comparisons of scab severity with soil chemical properties (Table 5.1).

1995 scab survey soils

Sample number	Tubers with some scab (%)	Number of tubers	Soil properties											Millieqivalents of exchangeable cations/100g						
			LOI%	pH	E.C. µS/cm	P ppm	K ppm	Ca ppm	Mg ppm	Mn ppm	Zn ppm	Cu ppm	B ppm	Kjeldahl N %	K	Ca	Mg	K+Ca	K+Mg	Ca+Mg	K+Ca+Mg
1	16.7	6	14.9	5.3	210	32	890	2220	380	920	6.4	7.0	1.6	0.25	2.28	11.08	3.13	13.35	5.40	14.21	16.48
2	0.0	3	16.1	6.7	110	29	280	3790	370	140	2.6	4.0	1.9	0.23	0.72	18.91	3.05	19.63	3.76	21.96	22.67
3	25.0	3	21.3	6.1	115	12	210	3450	190	55	1.9	4.4	2.2	0.23	0.54	17.22	1.56	17.75	2.10	18.78	19.32
4	33.3	3	17.8	6.6	160	13	650	4240	640	330	1.7	4.2	2.0	0.24	1.66	21.16	5.27	22.82	6.93	26.43	28.09
5	20.0	5	19.7	6.0	120	19	260	3280	170	96	2.1	5.2	2.2	0.39	0.67	16.37	1.40	17.03	2.06	17.77	18.43
6	0.0	4	15.9	6.3	130	27	280	3200	300	82	1.9	2.7	2.2	0.22	0.72	15.97	2.47	16.68	3.19	18.44	19.15
7	0.0	12	17.8	4.6	330	21	250	1200	200	200	3.5	2.3	2.7	0.37	0.64	5.99	1.65	6.63	2.29	7.63	8.27
8	22.2	9	15.6	5.8	115	29	310	2330	390	250	2.5	5.0	3.0	0.19	0.79	11.63	3.21	12.42	4.00	14.84	15.63
9	88.9	9	20.3	5.6	140	15	290	2390	140	38	1.5	3.7	2.4	0.11	0.74	11.93	1.15	12.67	1.89	13.08	13.82
10	50.0	4	12.8	6.4	85	20	310	2370	530	83	2.1	1.7	2.4	0.26	0.79	11.83	4.36	12.62	5.16	16.19	16.98
11	33.3	3	20.6	5.1	125	13	240	2050	160	51	2.2	4.1	2.3	0.44	0.61	10.23	1.32	10.84	1.93	11.55	12.16
12	0.0	3	18.5	5.6	245	34	470	2460	340	94	1.4	2.4	2.5	0.37	1.20	12.28	2.80	13.48	4.00	15.07	16.28
13	50.0	4	19.9	6.1	155	16	520	4130	690	190	1.7	4.1	2.3	0.43	1.33	20.61	5.68	21.94	7.01	26.29	27.62
14	0.0	9	23.1	4.2	325	14	210	1370	130	130	2.8	2.5	3.4	0.49	0.54	6.84	1.07	7.37	1.61	7.91	8.44
15	0.0	9	22.6	4.8	175	9	170	2140	370	44	1.6	2.0	3.7	0.45	0.43	10.68	3.05	11.11	3.48	13.72	14.16
16	0.0	5	16.9	6.1	465	16	340	2880	910	2	2.1	1.8	2.6	0.52	0.87	14.37	7.49	15.24	8.36	21.86	22.73
17	0.0	2	15.0	6.4	145	26	360	2870	730	120	2.5	2.6	3.0	0.32	0.92	14.32	6.01	15.24	6.93	20.33	21.25
18	0.0	6	20.8	4.7	195	13	310	1050	170	1200	1.1	8.8	2.8	0.40	0.79	5.24	1.40	6.03	2.19	6.64	7.43
19	0.0	7	19.8	5.1	125	13	200	1900	220	150	1.5	2.5	3.1	0.44	0.51	9.48	1.81	9.99	2.32	11.29	11.80
20	20.0	5	16.1	5.7	130	32	390	2300	360	210	2.5	3.6	3.0	0.31	1.00	11.48	2.96	12.47	3.96	14.44	15.44
21	33.3	6	17.3	6.3	95	16	430	3470	530	190	2.3	5.2	2.5	0.33	1.10	17.32	4.36	18.42	5.46	21.68	22.78
22	0.0	6	24.1	4.7	180	13	170	780	130	72	1.9	5.1	3.1	0.46	0.43	3.89	1.07	4.33	1.50	4.96	5.40
23	0.0	6	12.6	5.0	150	20	280	1320	120	520	1.8	2.0	2.7	0.29	0.72	6.59	0.99	7.30	1.70	7.57	8.29
24	0.0	6	4.7	4.8	135	26	220	1060	120	380	1.3	1.5	1.5	0.14	0.56	5.29	0.99	5.85	1.55	6.28	6.84
25	0.0	6	19.0	5.3	205	24	350	2310	160	110	1.2	1.1	3.0	0.39	0.90	11.53	1.32	12.42	2.21	12.84	13.74
26	28.6	7	13.4	5.0	200	11	360	2230	240	150	1.2	2.1	3.1	0.20	0.92	11.13	1.98	12.05	2.90	13.10	14.02
27	0.0	7	13.3	5.6	175	43	500	2750	310	710	5.6	5.1	2.3	0.28	1.28	13.72	2.55	15.00	3.83	16.27	17.55
28	28.6	7	13.6	5.5	230	62	250	2890	170	220	2.5	3.0	2.6	0.33	0.64	14.42	1.40	15.06	2.04	15.82	16.46
29	0.0	7	18.4	4.8	175	7	180	1560	110	47	0.8	2.9	3.7	0.33	0.46	7.78	0.91	8.24	1.37	8.69	9.15
30	28.6	7	19.1	5.9	270	20	270	3030	360	180	5.2	4.8	2.8	0.40	0.69	15.12	2.96	15.81	3.65	18.08	18.77
31	0.0	7	17.8	6.0	180	20	230	2960	300	110	3.5	4.1	2.4	0.36	0.59	14.77	2.47	15.36	3.06	17.24	17.83
32	0.0	4	14.4	6.5	120	17	330	4110	250	170	1.8	3.5	2.7	0.33	0.84	20.51	2.06	21.35	2.90	22.57	23.41
33	0.0	9	24.2	5.2	170	6	85	1780	220	290	1.4	5.0	3.1	0.36	0.22	8.88	1.81	9.10	2.03	10.69	10.91
34	0.0	5	13.1	5.0	185	10	160	1150	58	9.9	1.2	0.3	2.8	0.29	0.41	5.74	0.48	6.15	0.89	6.22	6.63
35	33.3	6	12.6	5.2	170	19	420	1830	360	980	4.1	6.6	2.7	0.26	1.07	9.13	2.96	10.21	4.04	12.09	13.17
36	100.0	11	22.1	5.2	230	12	430	2920	210	140	2.1	4.2	2.4	0.46	1.10	14.57	1.73	15.67	2.83	16.30	17.40

Table 5.1 Chemical properties of 1995 scab survey soils and scab severity associated with those soils assessed in a pot trial.

Also included in this table are levels of K, Ca and Mg calculated as milliequivalents per 100g of soil. Sample 16 was excluded from the statistical analyses as it was a substantially different soil type (being a sandy loam).

Multiple linear regressions (Table 5.2) revealed little relationship between single soil properties and scab severity. The best fitting regressions were weak correlations with calcium (p=0.065) and potassium (p=0.105) levels. Combining the K and Ca data as milliequivalents/100g soil produced a slightly improved regression (p=0.055). Combined milliequivalents of the three main exchangeable cations K, Ca and Mg [Meq. (K+Ca+Mg)] gave p=0.064. An R^2 value of 0.10 for this variable indicates that it accounted for only 10% of the observed variation in scab severity.

Table 5.2 Regressions of measured variables with scab severity[#]

Variable	R^2	p value for regression
LOI%	0.02	0.456
pH	0.04	0.246
Conductivity	0.01	0.545
P	0.01	0.571
Kjeldahl N%	0.02	0.436
Mn	0.01	0.560
Cu	<0.01	0.959
Zn	0.03	0.347
B	0.06	0.142
Meq K[§]	0.08	0.105
Meq Ca	0.10	0.065
Meq Mg	0.03	0.298
Meq (K+Ca)	0.11	0.055
Meq (K+Mg)	0.05	0.202
Meq (Ca+Mg)	0.09	0.072
Meq (K+Ca+Mg)	0.10	0.064

[#] Scab severity was measured as percentage of tubers with some scab.

[§] Meq = milliequivalents per 100g of soil

It was observed that scab was absent from those soils where Meq.(K+Ca+Mg) was equal to or less than twelve units. This represented ten samples out of 35. Analysis of this data using Fisher's Exact Test with a 2 x 2 contingency table (Figure 5.2) revealed a very significant effect of Meq.(K+Ca+Mg) on presence/absence of scab, with

the probability of obtaining the observed distribution or a more extreme one being p=0.0005.

	Meq. (K+Ca+Mg) ≤12	>12	Total	Significance
Soils with scab absent.	10	9	19	
Soils with scab present.	0	16	16	
Total	10	25	35	p=0.0005

Figure 5.2 Fisher's Exact test contingency table with threshold for combined milliequivalents of exchangeable Ca^{2+}, Mg^{2+}, and K^{+}= 12.0. p = the probability of obtaining the observed distribution or a more extreme one.

A similar trend was noted for soil pH. Scab was not present in 9 out of 10 samples with a pH of 5.0 or less while scab was present for 15 out of 25 samples with pH greater than 5.0. The probability of obtaining this distribution or a more extreme one was p=0.009 (Figure 5.3A). At a pH threshold of 5.2 the relationship becomes less significant (p=0.052: Figure 5.3B).

A)

	pH≤5.0	pH>5.0	Total	Significance
Soils with scab absent.	9	10	19	
Soils with scab present.	1	15	16	
Total	10	25	35	p=0.009

B)

	pH≤5.2	pH>5.2	Total	
Soils with scab absent.	11	8	19	
Soils with scab present.	4	12	16	
Total	15	20	35	p=0.052

Figure 5.3 Fisher's exact test contingency tables with **A)** pH threshold =5.0 and **B)** pH threshold = 5.2. p = the probability of obtaining the observed distribution or a more extreme one.

The relationship between combined level of the exchangeable cations K^+ Ca^{2+} and Mg^{2+} with pH in these soils is plotted in Figure 5.4. A regression line fitted through this data has an R^2 of 0.773 with a probability of $p=3.68x10^{-12}$. The dominant effect of calcium (among these ions) on pH in the soils is shown in a plot of pH against Meq. Ca (Figure 5.5) where the fitted regression had an R^2 of 0.758 with a probability of fit being $1.09x10^{-11}$.

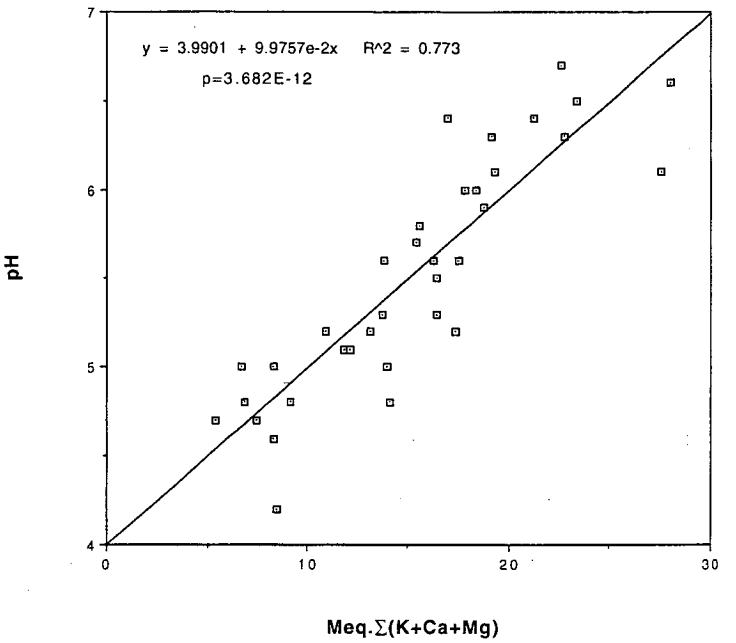


Figure 5.4 Plot of pH against combined milliequivalents of exchangeable Ca^{2+}, Mg^{2+}, and K^+ for 36 soils from the 1995 potato scab survey.

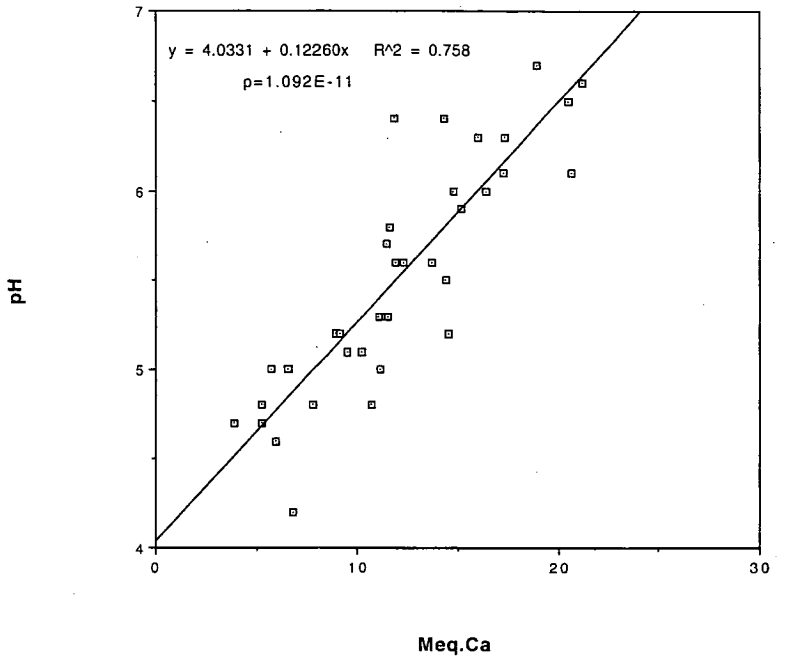


Figure 5.5 Plot of pH against exchangeable Ca^{2+} for 36 soils from the 1995 potato scab survey.

Re-examination of similar survey data from 1994 (Appendix 3.2) revealed indications of similar trends in the relationships between scab severity, exchangeable Ca, K and Mg, and soil pH although the method of measuring scab was different in this case. A scab index (SI) based on the method of Bjor and Roer (1980) had been used, with values ranging from 0 to 880 and the distribution skewed toward smaller values (differences in scab levels may be partly due to difference in agronomic practices between sites). When looking at presence or absence of scab no strong correlations were found. If however, scab severity is grouped as either being mild to nil (SI≤20) or moderate to severe (SI>20) relationships between disease and Meq.(K+Ca+Mg) or pH are observed. If considering a threshold of 12 for Meq.(K+Ca+Mg) as before (Figure 5.6) is not significant at p=0.093.

	Meq. (K+Ca+Mg) ≤12	>12	Total	Significance
Soils with scab index ≤20	15	6	21	
Soils with scab index >20	4	6	10	
Total	19	12	31	p=0.093

Figure 5.6 Fisher's exact test contingency table with threshold for combined milliequivalents of Ca^{2+}, Mg^{2+}, and K^{+}=12.0, and with a scab index threshold of 20. p= the probability of obtaining the observed distribution or a more extreme one.

If alternatively a threshold of 9.5 is chosen, the significance improves to p=0.002 (Figure 5.7)

	Meq. (K+Ca+Mg) ≤9.5	>9.5	Total	Significance
Soils with scab index ≤20.	12	9	21	
Soils with scab index >20.	0	10	10	
Total	12	19	31	p=0.002

Figure 5.7 Fisher's exact test contingency table with threshold for combined milliequivalents of Ca^{2+}, Mg^{2+}, and K^{+}=9.5, and with a scab index threshold of 20. p= the probability of obtaining the observed distribution or a more extreme one.

With a pH threshold of 5.5 the pH data was also marginally significant at p=0.046 (Figure 5.8)

In contrast to the 1995 results, regressions for the 1994 (DPIWE) data (Table 5.3) reveal that for this data set the correlation of scab [calculated as log(scab index+1)] with pH although not significant (at p=0.057) was greater than with any of the exchangeable cations. Additionally for this data set the regression between pH and exchangeable calcium had $R^2 = 0.564$ with a probability $p = 1.14 \times 10^{-6}$ and the regression between pH and Meq (K+Ca+Mg) gave $R^2 = 0.541$

with p= 2.40×10^{-6} once again showing the close relationship between exchangeable cations (particularly calcium) and soil pH.

	pH≤5.5	pH>5.5	Total	Significance
Soils with scab index ≤20.	10	11	21	
Soils with scab index >20.	1	9	10	
Total	11	18	31	p=0.046

Figure 5.8 Fisher's exact test contingency table with threshold for pH=5.5, and with a scab index threshold of 20. p= the probability of obtaining the observed distribution or a more extreme one.

Table 5.3 Regressions of 1994 data with log(SI+1)[#]

Variable	R^2	p value for regression
LOI%	0.10	0.088
pH	0.12	0.057
Conductivity	0.06	0.194
P	<0.01	0.726
Mn	<0.01	0.743
Cu	<0.01	0.786
Zn	<0.01	0.950
B	0.08	0.115
Meq K[§]	0.02	0.482
Meq Ca	0.06	0.202
Meq Mg	0.02	0.493
Meq (K+Ca)	0.06	0.185
Meq (K+Mg)	0.03	0.351
Meq (Ca+Mg)	0.06	0.191
Meq (K+Ca+Mg)	0.06	0.177

[#] Scab index (SI) based on the method of Bjor and Roer (1980).

[§] Meq = milliequivalents per 100g of soil.

5.4 Discussion

Results of this study appear to indicate a relationship between scab severity, soil pH and the levels of exchangeable Ca, Mg and K in krasnozem soils . Specifically they indicate that the probability of scab is reduced at low pH or low combined levels of the measured exchangeable cations. Effect of low pH in reducing scab is hardly a new finding. Soil pH below about 5.2 is generally suppressive to scab (Waksman, 1921).

Also, strong correlations were found between a combined measure of these exchangeable cations and soil pH, and between exchangeable calcium and pH. In light of the findings of others (Lambert and Manzer, 1991), the observation of a close relationship between exchangeable cations (particularly calcium) and pH, lends support to the conclusion that the observed effects on scab in this study are most likely due to pH. Findings from the 1994 DPIWE data set also show a better correlation of scab with soil pH than with exchangeable Ca, Mg and K although both correlations were weak.

In the isolates study, Tasmanian *S. scabies* strains were found to grow at pH 4.5, with some strains growing at 4.0. The current study provides evidence that soil factors other than pH may be important in determining the soil pH threshold at which scab disease can occur.

These results also show the robustness of the pH threshold associated with common scab disease. Elsewhere common scab has generally not been observed in soil below pH 5.2 (Waksman, 1921; Powelson *et al.*, 1993). In the current results and with the results of the 1994 survey a similar threshold has been independently determined by two separate methods. For the 1994 data, scab severity had been determined by a field assay, while with the 1995 data scab severity was determined using a glasshouse assay.

The results of the current study are indicative only, as scab levels measured in the glasshouse assessment were very mild. Linear regressions showed no strong correlation with scab of the measured soil properties. Further experimentation would still be required to determine if the measured effect is real, and if so to explore the underlying mechanisms.

One possible factor that may have influenced the results of this study is the prior storage for 12 months at 4°C of soil samples from the

1995 scab survey. It is unlikely that this storage would have significantly affected the soil chemistry (L. Sparrow, pers. com.). The effect on soil microbiology is largely unknown as there have been few studies on the effect of refrigeration temperatures on the death rate of microorganisms (Parkinson *et al.*, 1971). However, low temperatures reduce microbial activity while not necessarily causing death of microorganisms.

Pooling of scab data for pathogen inoculated and uninoculated treatments in the 1995 dataset could also be another source of error. This data was pooled due to the very limited number of replicates and low level of scab observed, and was done in order to increase the sample size. For such pooling to be valid it must be assumed that there was sufficient inoculum already in the uninoculated pots for some scab disease to develop. It is considered unlikely that scab inoculum was not present in these soils as they were agricultural soils that had been previously used for potato cropping. The appearance of scab lesions was similar on tubers from both inoculated and uninoculated pots. Concurrence of the pH threshold found with this survey with that from the 1994 data and that of others may be evidence that effects of sample storage and pooling of data may not have been significant.

6 Green Manures

6.1 Introduction

Increasingly, Tasmanian potato growers are including green manures in their crop rotations. These are grown as a cover crop over winter, and turned-in prior to spring sowing of the potato crop. There are anecdotal reports of green manures being associated with both increased and decreased incidence of common scab. The Tasmanian Department of Agriculture (now DPIWE) has previously recommended (Anon., 1954) digging in of green manures to inhibit common scab development. However the effects of green manures on common scab are largely unknown and there appears to have been no previous study on the effects of green manures on common scab under Tasmanian cropping conditions.

Green manuring is a means by which either the soil humus content or soil nitrogen can be increased (Russell, 1973). In association with green manure addition, changes in soil microbiota can occur (Rouatt and Atkinson, 1950). Various studies on green manures elsewhere have suggested that scab suppressiveness of green manure treatments may be due to stimulation of antagonistic micro-organisms (Millard and Taylor, 1927; Rouatt and Atkinson, 1950; Weinhold and Bowman, 1968). With this in mind it was considered an interesting possibility that any suppressiveness might be supplemented by inoculating a suitable antagonist strain into the green manure material at the time of application to soil.

The goals of this green manures study were to 1) determine under glasshouse conditions whether green manures could affect scab severity in a krasnozem soil; 2) determine whether inoculation of the green manure with a known scab antagonist could have additional effects on scab severity; 3) validate under commercial field conditions, any effects found in glasshouse experiments.

6.2 Green-manures pot trials

6.2.1 Green manures trial 1 - Evaluation of effects of green manures and a streptomycete antagonist on incidence of common scab in a krasnozem soil

6.2.1.1 Introduction

The aim of this pot trial was to evaluate the effects of a legume and cereal as green manures in combination with a known streptomycete antagonist on scab severity and potato growth in a krasnozem soil. Treatments were as shown in Table 6.1. and were replicated three times. Green manures were applied at the rate of 1 kg fresh weight per 20 kg dry soil.

Table 6.1) Treatments included in green manures trial 1

Green Manure	Inoculum
None	None
Broad Bean	None
Triticale	None
None	Strain #23
Broad Bean	Strain #23
Triticale	Strain #23

6.2.1.2 Pot trial establishment

Krasnozem soil was collected from a field near Deloraine, Tasmania in which severe common scab had been recorded for a potato crop in the previous season. Soil was mixed to a uniform consistency before use.

Mature plants of triticale (*x Triticosecale* Wittmac) and broad bean (*Vicia faba* L.) were harvested from a field site and stored in plastic bags at 4°C for two weeks. Vegetation was chopped with secateurs into pieces 10 cm or less in length prior to adding to soil.

The antagonist (*S. scabies* strain #23) was grown on YME agar. Spores scraped from the surface of colonies were suspended in sterile water and added at 1.8×10^7 spores per gram of soil. Where antagonist was used in combination with a green manure this was added to the green manure twelve hours prior to incorporation into the soil.

Spore suspension was poured onto vegetation in a plastic bag and mixed-in by shaking. For the soil-only control, antagonist was applied to a small amount of soil which was then thoroughly mixed with the remainder of soil in that pot.

Pots consisted of plastic crates with dimensions 34 x 31.5 cm wide by 30 cm high. To enable easy drainage, holes were drilled in the bottom of the crates in to which a 2 cm layer of coarse gravel was placed and separated from the overlying soil by polypropylene mesh. Soil was distributed in equal amounts of 19.5 kg (dry weight) to each of 18 pots.

6.2.1.3 First Sowing

Four weeks after applying green manures, pots were each sown with 9 rooted sprouts (8 cm average length) taken from tubers of potato cv. Russet Burbank. Although nine plants were sown per pot, each pot was treated as a single sampling unit with results calculated on a per plant basis.

Pots were arranged in two randomised blocks. Two tensiometers were placed in one of the pots to monitor soil moisture at the bottom and near the soil surface in that pot (Figure 6.1). Watering was the minimum amount for healthy plant growth, with all pots receiving an equal amount of water.

Soil core samples were taken for cellulase assays at time of sowing and at time of tuber initiation. Pooled samples for each pot consisted of three cores taken to a depth of 5 cm with a 12 mm cork borer. Between pots the cork borer was washed with water to remove soil then wiped with ethanol and allowed to dry. Samples for enzyme assays were stored at room temperature in plastic bags and assayed within three days from the time of collection.

Figure 6.1 Green manures pot trial 1. Mature potato plants at 10 weeks after first sowing. Two tensiometers had been placed in one pot (left) to monitor soil moisture. Treatments were: C, control; L, triticale; H, broad bean; IC, control + strain #23; IL, triticale + strain #23; HI, broad bean + strain #23.

Plants were harvested at 12 weeks after sowing. Soil samples (300 g) were collected at harvest for chemical analysis and microbial counts. Electrical conductivity, pH and Kjeldahl nitrogen were determined for all replicates while pooled treatment samples were used in all other assays. Measurement of loss on ignition, exchangeable cations (K^+, Ca^{2+} and Mg^{2+}), phosphate, trace elements(Mn, Zn, Cu, and B) and N% (Kjeldahl) was determined as described previously (The Soils, 5.2.2).

Actinomycete cfu from soil samples were counted on GAT agar and CMCA agar (General Methods, 3.4.1). Comparative counts were made of other bacteria and fungi growing on these media.

Scab severity in this experiment was determined as percentage of tuber surface area affected. Assessment involved measuring all lesions individually with a vernier caliper and calculating the total surface area affected for each tuber. This figure was divided by the tuber surface area, which was calculated from tuber dimensions using Equation 1 (approximating calculation of surface area of a sphere). Tubers with a volume of $5cm^3$ or less (calculated using equation 2) were excluded from

measurements. Average percent scab was then calculated for each pot. Although a precise measure of scab severity was obtained, this was a very tedious and time consuming method and was not repeated in subsequent experiments. In remaining pot and field experiments scab was scored as number of lesions per tuber.

Equation 1: $$Area = 4\pi\left(\frac{abc}{8}\right)^{\frac{2}{3}}$$

Where a, b and c are length, width and breadth respectively of tubers, measured in centimetres.

Equation 2: $$Volume = \frac{4}{3}\pi\frac{abc}{8}$$

Where a, b and c are the same as for Equation 1.

For statistical analysis of percentage scab data, a log transformation was applied.

6.2.1.4 Second sowing

Pots were left outside the glasshouse to simulate fallow conditions for 20 weeks over the autumn/winter period. Except for occasional hand weeding no additional treatment was required during this time. At the end of the fallow period, pots were returned to the glasshouse and resown to determine continuity of effect over an additional season.

Eye cores from Russet Burbank tubers were sown at nine per pot, planted to a depth of 3 cm. Pots were arranged in one randomised block. Plants were harvested at 12 weeks after sowing. At harvest, tubers weighing ≥ 2.0 g were assessed for scab severity and yield, while soils were assessed for pH and soil conductivity but not enzyme activity.

6.2.2 Second green manures trial - Further evaluation of effects of green manures and streptomycete antagonists on common scab in a krasnozem soil

6.2.2.1 Introduction

Unlike the first pot trial, green manure plants in this trial were grown in the soils into which they were later mixed. Three green manure crops were compared, a legume (lupin, *Lupinus angustifolius* L.) and cereal (rye, *Secale cereale* L.) were included as in the first trial, plus a brassica (mustard, *Brassica juncea* L.). A "fallow" control was included as before. Included as an additional factor, as in the first trial, microbial inoculum was applied with the green manure. Two streptomycete antagonists, strains 2/2-5 and 25/2 were used. Treatments were as in Table 6.2 (with 5 replicates).

Table 6.2 Green manures trial 2 - Treatments

Green Manure Species	Antagonist
None	None
None	Strain 2/2-5
None	Strain 25/2
Lupin	None
Lupin	Strain 2/2-5
Lupin	Strain 25/2
Rye	None
Rye	Strain 2/2-5
Rye	Strain 25/2
Mustard	None
Mustard	Strain 2/2-5
Mustard	Strain 25/2

6.2.2.2 Pot trial establishment

The soil (a krasnozem collected from a site near Devonport, Tasmania) was amended in attempt to make it more scab conducive, by addition of pathogen inoculum and application of 6.8 gl^{-1} limil (calcium hydroxide) to increase pH. Pathogenic *S. scabies* strain #32 was grown for three weeks on spread plates of YME. Sporulating cultures and associated media were mixed with a small amount of soil which was then mixed thoroughly with the remaining soil giving a final inoculum load of 3.6 $x10^5$ spores ml^{-1} of soil.

Green manure crops were sown at the recommended field rate of equivalent to 30 g of seed per square metre into the prepared soil in 20 cm (diameter) pots. Plants were grown under glasshouse conditions (Figure 6.2) and vegetation harvested at flowering time (at 3 months after sowing; Figure 6.3).

Figure 6.2 Growth of plants of rye, mustard and lupin to be subsequently used as green manures in the second green manures pot trial.

Figure 6.3 Green manure plants immediately prior to harvest. These were (from left to right): lupin, mustard and rye.

Vegetation was chopped into pieces ≤10 cm and bulked for each green manure type before re-application to pots at equivalent to 22 g dry weight per pot (6.4 gkg^{-1} soil). A small amount of additional lupin material was added to make the required weight.

Inoculum of strains 2/2-5 and 25/2 were grown for two weeks on YME. Spores scraped from plates were suspended in sterile water and applied at a rate equivalent to 10^6 spores ml^{-1} soil.

Green manures and inoculum were applied to soil in the following way. Soil was removed from pots to a depth of 10 cm and bulked for each treatment (5 pots). Green manures were then mixed uniformly with soil before returning it equally to the pots. Where inoculum was added, this was applied first to the green manures and mixed thoroughly before mixing into the soil. Where inoculum was applied to controls without green manures this was first applied to a small amount of soil, which was then uniformly mixed-in with the remainder of the soil.

6.2.2.3 First sowing

Pots were each sown with three Russet Burbank eye cores at the time of incorporation of the green manures. Pots were arranged in one randomised block (Figure 6.4). Plants were harvested 13 weeks after sowing. At harvest, soil samples were pooled for each treatment and analysed for pH, electrical conductivity, cellulase and FDA hydrolysis activity. Scab severity and tuber yield were determined.

6.2.2.4 Second sowing

After a 25 week fallow period the pots were resown with cultivar Kennebec, a cultivar more susceptible to common scab than Russet Burbank. Plants were harvested after 14 weeks. Other experimental details were the same as in the first sowing except that soil measurements were made for individual replicates and that tuber numbers were not determined.

Figure 6.4 Green manures pot trial 2 - first sowing. Potato cultivar was Russet Burbank.

6.2.3 Effects of *S. scabies* and *S. violaceusniger* strains on growth of green manure plants

The purpose of this pot trial was to determine whether the two possible pathogen types identified in the isolates studies could have an appreciable effect on growth of green manure crops. Three green manure crops were grown in potting mix inoculated with representative strains of *S. scabies* and *S. violaceusniger*. Treatments were as shown in Table 6.3.

Table 6.3 Treatments in pot trial to evaluate the effects of *S. scabies* and *S. violaceusniger* on growth of green manure crops.

Green Manure Crop	Inoculum
Lupin	*S. scabies* strain 1/2-3
Lupin	*S. violaceusniger* strain 46/1A-1
Lupin	None
Mustard	*S. scabies* strain 1/2-3
Mustard	*S. violaceusniger* strain 46/1A-1
Mustard	None
Rye	*S. scabies* strain 1/2-3
Rye	*S. violaceusniger* strain 46/1A-1
Rye	None

Plants were grown in a potting mix which consisted of a 4:5:1 mixture of peat/coarse-sand/perlite amended with $10gl^{-1}$ dolomite, 3.125 gl^{-1} limil (calcium hydroxide), 6.25 gl^{-1} 'Osmocote'™ slow release fertilizer (NPK= 10:4.8:15) and 0.31 gl^{-1} trace elements. Strains were grown for 26 days on SAY/vermiculite medium (Appendix 1.1.16) 15 ml of which was applied evenly to surface of soil in each pot. Seed was sown into 16 cm pots at rate of: lupin, 3.5 g/pot; rye, 2.5 g/pot; and mustard, 2.0 g/pot, with five replicate pots per treatment. Pots were initially grouped according to inoculum type and spaced 20 cm apart to minimise cross contamination (Figure 6.5). Following seedling establishment, pots were randomised within each crop type at 20 cm pot spacing.

Plants were harvested at the time of flowering and dry weights of above-ground vegetation and, where possible, root mass was measured. Plate counts were made of soil actinomycetes.

Figure 6.5 Pot trial to evaluate the effects of *S. scabies* and *S. violaceusniger* strains on growth of green manure species. Shown are (from front to back): mustard, rye and lupin. Pots were later randomised within each plant type.

6.1.3 Field Validation of Green Manure Studies

6.1.3.1 Introduction

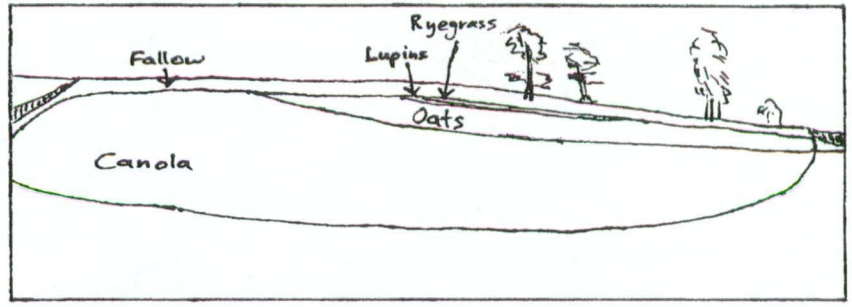
Following on from the glasshouse studies, trials were conducted on two potato grower's properties to evaluate the effects of green manures on scab severity, potato yield and soil properties under commercial conditions. The factors assessed were the same as previously studied in the first two pot trials. Changes in soil properties over time were also assessed over four sample times over an eight month period, including twice during the green manure growth phase and twice during the potato growth phase. No microbial inoculum was applied to the sites.

6.2.4.2 Sites

Two suitable sites on potato grower's properties were available for study, these being at Springfield in Tasmania's north east and at Deloraine in the central north. At the Springfield site (Figure 6.6) the soil type, although considered a krasnozem contained some sand and the land had a gentle (around 1 in 14) uniform slope down toward the north. The land at the Deloraine site (Figure 6.8) was flat and the soil a typical krasnozem. The growers were responsible for sowing and maintainance of the green manure crops and subsequent potato crops as part of their normal commercial operations. For simplicity of grower operations, green manures were sown in single adjacent strips instead of multiple blocks (Figures 6.7 & 6.9). It is recognised that the consequent lack of block replication in green manure types across each site places limits on the reliability of the results, for which it is necessary that each site needed to be uniform throughout. Green manure crops sown at both sites were oat (*Avena sativa* L.), bitter blue lupin (*Lupinus angustifolius* L.) and ryegrass (*Lolium perenne* L.) with seed for both sites being from the same seed lots. Additionally, canola (*Brassica napus* L.) was sown at the Springfield site. Fallow areas were established at both sites. At Deloraine this involved leaving a cultivated strip unsown. At Springfield, the fallow strip was sprayed with herbicide but left uncultivated. Both sites were sown with potato cultivar Russet Burbank and were irrigated during dry weather.

Figure 6.6

View of the Springfield site early in the green manure growth phase (11 June 1997)



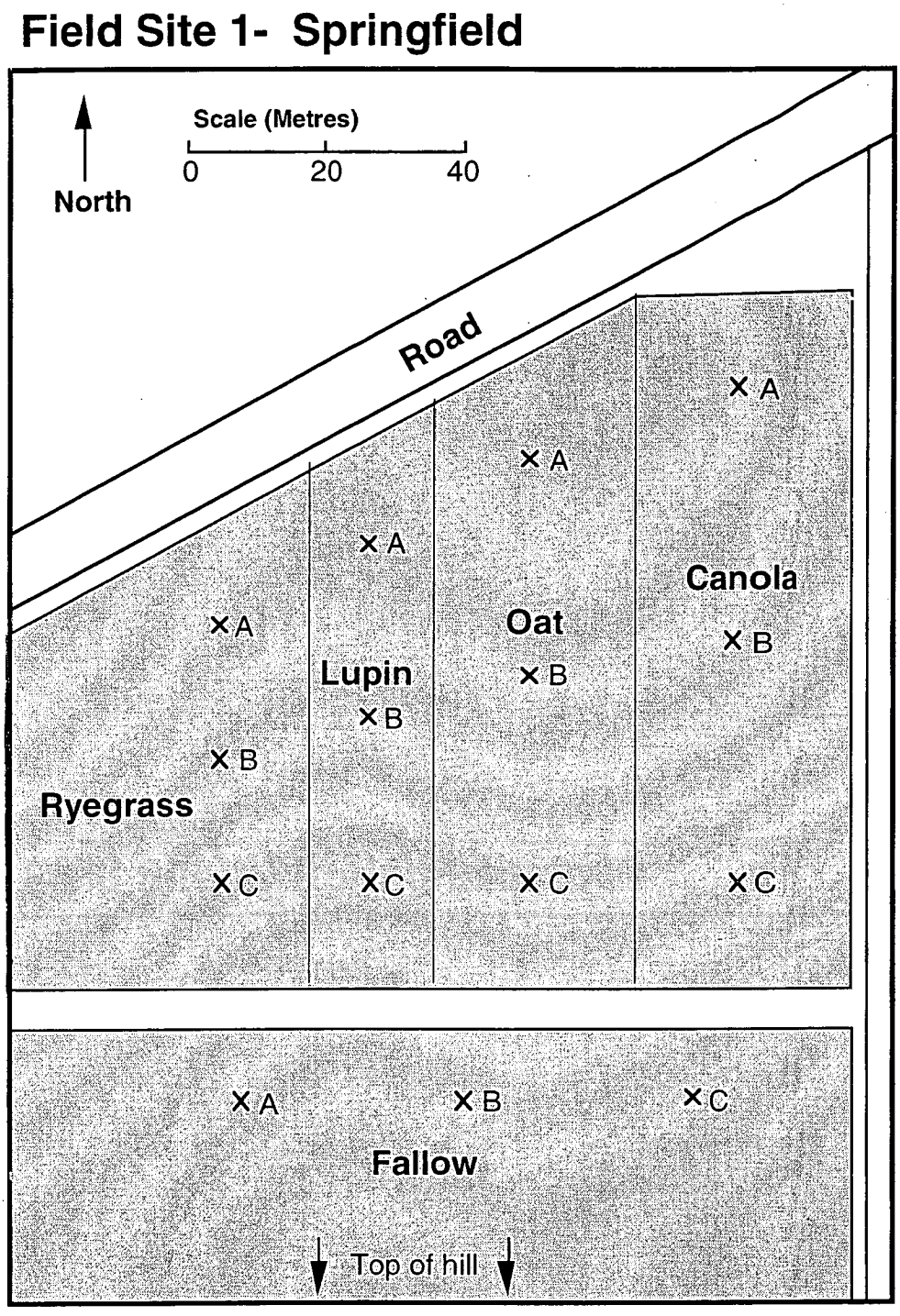
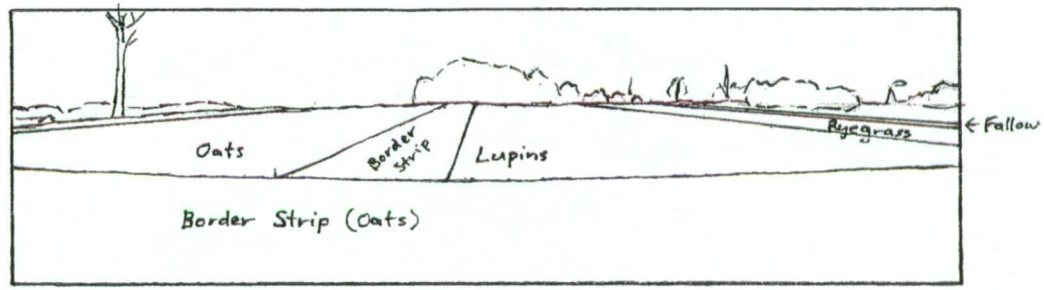


Figure 6.7 Layout of Springfield trial site.

Figure 6.8

View of the Deloraine site early in the green manure growth phase (19 June 1997)



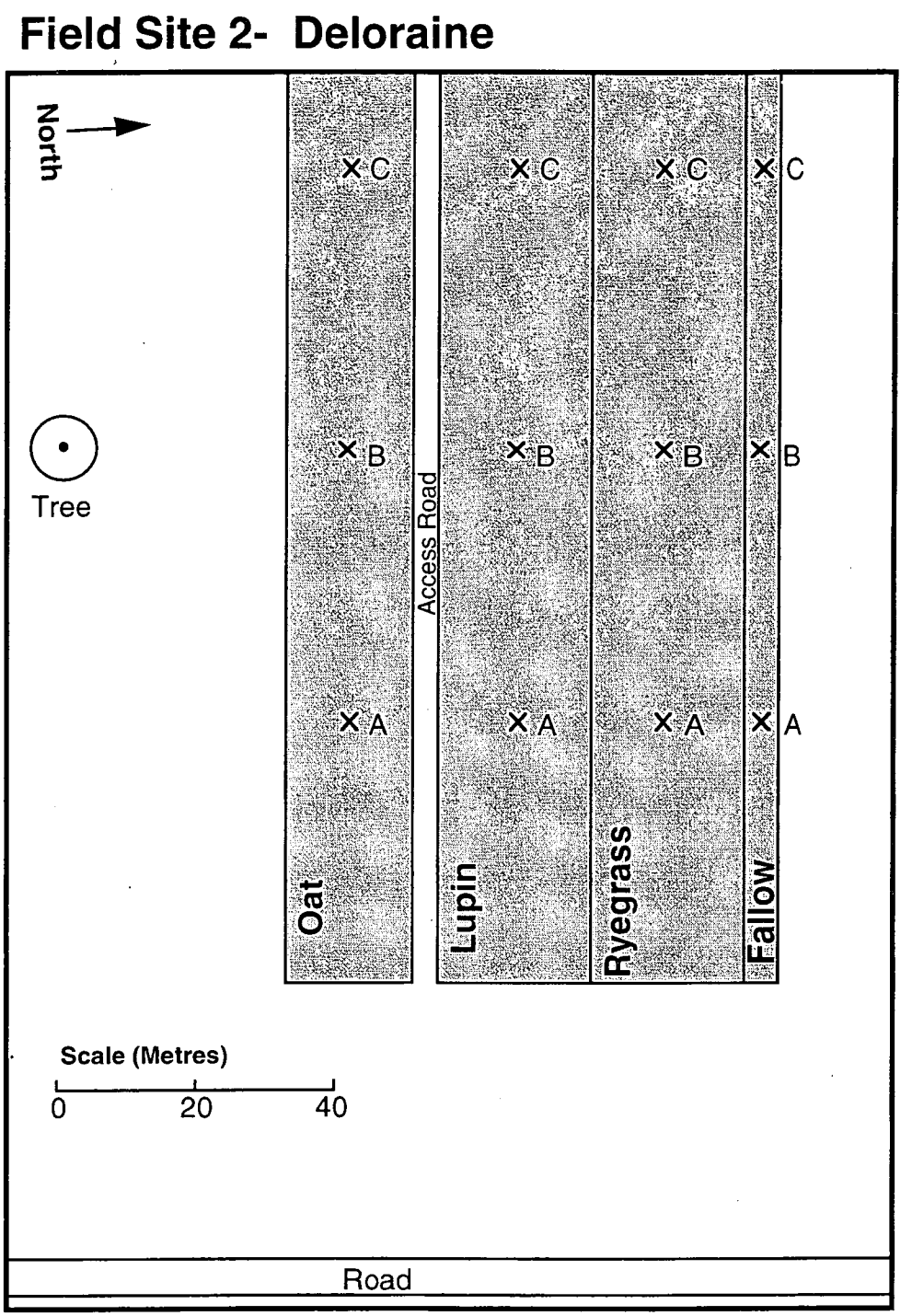


Figure 6.9 Layout of Deloraine trial site.

6.2.4.3 Experimental procedure

The same procedure was followed for sampling of each site.

Three replicate sampling points were located within each green manure or fallow strip. (marked **x** in figures 6.7 and 6.9). Soil was sampled from all sampling points at both sites at the following approximately equally spaced times:

- after establishment of green manure plots, when vegetation was still sparse
- when green manure crops were mature and prior to turning-in
- at around 2 weeks after sowing potatoes, when plant emergence had not yet occurred
- when potato plants were mature and flowering.

These samples each consisted of three pooled soil cores, to a depth of 12 cm, randomly collected within a range of 2 m of the sample point.

Vegetation was sampled prior to turning in. Two samples collected from randomly placed 1860 cm^2 quadrats were pooled for each green manure plot.

At harvest, four adjacent potato plants were independently sampled at each of the three sample points on each green manure strip (giving twelve plants sampled per treatment). All tubers were collected from each plant sampled. Tubers were later counted, individually weighed and scored for scab severity.

Monthly rainfall and temperature data covering the experimental period was obtained from the Australian Bureau of Meteorology for a location (station 91219) 5 km north of the Springfield site. No corresponding data was available for any location close to the Deloraine site.

6.3 Results

For the majority of variables measured in the green manures experiments no significant differences were found between treatments. Where treatment differences were significant or the data considered otherwise noteworthy those results are highlighted here. Full numerical results of green manures experiments are presented in Appendices 2.3 to 2.11.

6.3.1 First green manures pot trial

6.3.1.1 Green manure dry matter and C:N determinations

The C:N ratio for triticale at 32.6 was over twice that for broad bean (12.3, Table 6.4). Dry matter added to soil was also greater for triticale (13.6 gkg^{-1}) than for broad bean (8.76 gkg^{-1}).

Table 6.4 Dry matter and C:N determinations for green manures pot trial 1.

	Green manure	
	Broad Bean	Triticale
Vegetation analyses		
Vegetation dry matter, (%)	17.1	27.1
Carbon content, (%).*	41.9	43.6
Nitrogen content, (%).*	3.40	1.34
C:N ratio	12.3	32.6
Green manure contributions to soil		
Vegetation dry matter added to soil (g/kg)	8.76	13.6
Dry matter per soil surface area in pots (kg/m^2)	1.60	2.53
Maximum carbon added to soil, (g/kg).	3.67	6.06
Maximum nitrogen added to soil, (g/kg).	0.30	0.19

* Percentage of green manure dry weight, determined by Carlo Erba 1108 CHNO-S analyser.

6.3.1.2 First sowing

6.3.1.2.1 Effects of antagonist strain #23 and interactions with green manure treatments

Inoculation with strain #23 was not associated with significant effects on any of the variables measured, with the sole exception of

mean tuber weight which was marginally lower in inoculated pots (21.3 g compared to 25.9 g for un-inoculated pots), this difference being significant at p=0.014.

There was no appreciable interaction between green manure treatments and inoculum level (Appendix 2.3).

6.3.1.2.2 Green manures and scab severity

Scabbing was mild in all treatments and mainly consisted of numerous small lesions. There was a discernible reduction ($P<0.05$) in scab severity in broad bean treatments relative to both the triticale and untreated control (Table 6.5).

Table 6.5 Effect of green manures on scab severity in trial 1, first sowing.

Green Manure	Percent of tuber surface area affected
None (control)	2.73 a[1]
Broad bean	1.47 b
Triticale	3.11 a
Standard Error	0.400

[1] Figures followed by the same letter are not significantly different at $p<0.05$ according to Fisher's Least Significant Difference Test. Each figure is the mean of 6 replicates with 9 plants per replicate.

6.3.1.2.3 Green manures, plant growth and tuber yield

Appearance of potato plants differed between treatments (Figure 6.1) in this pot trial. In comparison to the controls, plants that had received a broad bean green manure were greener while plants with the triticale manure tended to show slight chlorosis, especially at the later stages of growth. Nevertheless, mean tuber weights for both green manure treatments were approximately 20% greater than the control (Table 6.6).

Table 6.6 Effect of green manures on tuber yield in trial 1, first sowing.

Green Manure	Mean tuber weight, (g)
None (control)	20.31 a[1]
Broad bean	24.84 b
Triticale	25.50 b
Standard Error	1.389

[1] Figures followed by the same letter are not significantly different at $p<0.05$ according to Fisher's Least Significant Difference Test. Each figure is the mean of 6 replicates with 9 plants per replicate.

6.3.1.2.4 Soil pH, electrical conductivity and nitrogen

Green manures were associated with changes in these soil properties measured at harvest (Table 6.7). Soil pH was marginally but significantly ($p<0.01$), reduced in both green manure treatments. There was also a substantial increase in electrical conductivity of soil associated with both green manures ($p<0.01$) and a marginal but significant ($p<0.01$) increase in percentage of soil nitrogen (Kjeldahl) in the broad bean treatment. The elevation of nitrogen in green manure treatments was within the range that may be expected based on measurements of nitrogen content of the green manures (Table 6.4).

Table 6.7 Effect of green manures on soil properties measured at harvest of trial 1

Green Manure	Soil pH	Electrical conductivity, (µS/cm)	Kjeldahl nitrogen, (%)
None (control)	6.8 a[1]	65.4 a	0.24 a
Broad bean	6.6 b	141.0 b	0.25 b
Triticale	6.6 b	104.4 b	0.24 a
Standard Error	0.03	8.37	0.002

[1] Figures in each column followed by the same letter are not significantly different at $p<0.01$ according to Fisher's Least Significant Difference Test. Each figure is the mean of 6 replicates.

6.3.1.2.5 Levels of exchangeable ions in soil

Elemental analysis of soil samples (pooled for each treatment at harvest) revealed elevated levels of exchangeable potassium ($p<0.05$) and magnesium ($p<0.01$) in green manure treatments (Table 6.8). A similar trend indicated for exchangeable calcium was not statistically significant. Combined millieqivalents (per 100g soil) of K, Ca and Mg had increased from 17.8 in the control to 18.9 in both green manure treatments.

Presence of green manures had no effect on levels of exchangeable phosphorus, manganese, zinc, copper or boron in the soil (Appendix 2.4).

Table 6.8 Effect of green manures on exchangeable potassium magnesium and calcium in soil at harvest of trial 1

Green Manure	Exchangeable K, (ppm)[1]	Exchangeable Mg, (ppm)[1]	Exchangeable Ca, (ppm)[1]
None (control)	485 a*[2]	495 a	2500
Broad bean	630 b*	520 b	2605
Triticale	695 b*	520 b	2580
Standard Error	27.4	2.9	21.8

[1] Analysis performed by a commercial laboratory using standard methods. Extraction procedure used 1 part soil: 5 parts 1N ammonium acetate at pH 4.8.

[2] Figures in each column followed by the same letter are not significantly different at $p<0.01$ according to Fisher's Least Significant Difference Test. Each figure is the mean of 2 samples with each sample being a composite of soil from three pots.

6.3.1.2.6 Cellulase activity and green manure decomposition

Soil cellulase activity was measured at 30 and 70 days following mixing of green manures into the soil, corresponding with time of sowing potato seed and time of tuber initiation.

Cellulase activity had risen substantially ($p<0.001$) in both green manure treatments at 30 days (Figure 6.10) to around 250% of the reading for untreated soil.

At 70 days, cellulase activity in the broad bean treatments had dropped to a value no different to the untreated soil. Activity in the triticale treatments, however, remained higher than either of the other treatments ($p<0.001$).

At harvest some undecomposed triticale material remained in the soil, while broad bean material was no longer visible.

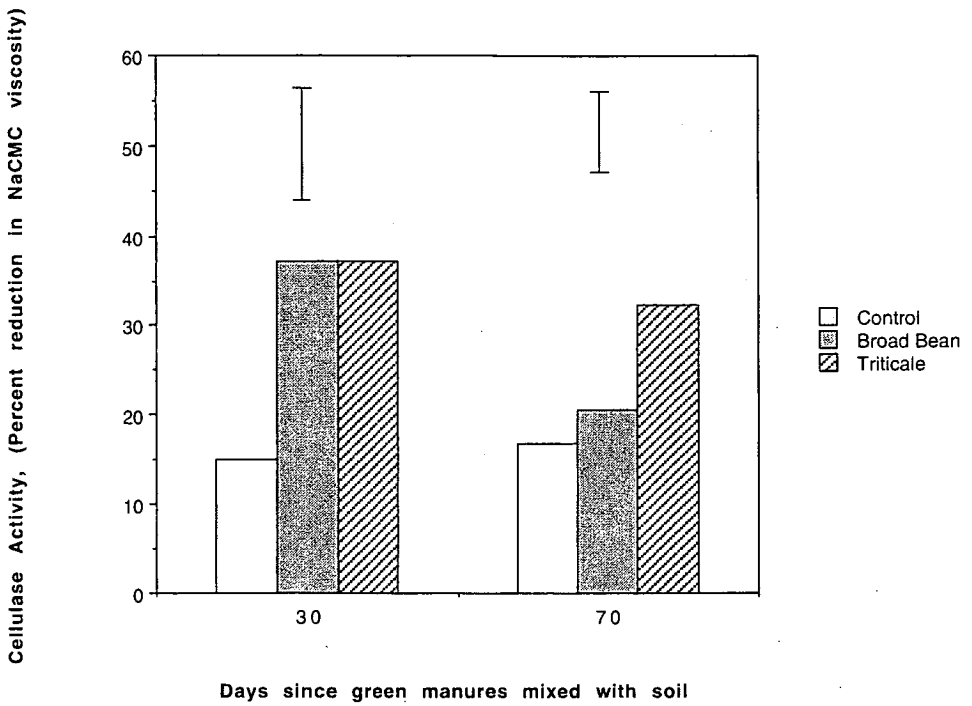


Figure 6.10 Soil cellulase activity at 30 days and 70 days since incorporation of green manures into soil, in green manures pot trial 1. Bars above graphs show $lsd_{(0.001)}$ for corresponding time period.

6.3.1.2.7 Plate counts

Plate counts of actinomycetes, other bacteria and fungi did not differ significantly between green manure treatments or between presence or absence of inoculum (Appendix 2.5). Counts of melanin producing and melanin non-producing actinomycetes on GAT medium averaged 1.5 $x10^5$ and 3.5 $x10^4$ colony forming units (cfu) respectively per gram of air dried soil. Actinomycetes counts on the CMCA agar averaged 3.8 $x10^5$ cfu per gram of air dried soil.

6.3.1.3 Second sowing

Scab development was mild, averaging 2.8 lesions per tuber, and did not differ significantly between treatments, when calculated as lesions per tuber, lesions per tuber weight or total lesion number.

Tuber yield was greater in the triticale treatments in terms of number and weight of tubers per plant (Table 6.9). Number of tubers per

plant was also marginally greater in treatments inoculated with strain #23 (count =2.6) compared to uninoculated treatments (2.1; p=0.032).

Soil electrical conductivity remained greater in green manure treatments than untreated soil at the time of harvest (Table 6.9).

Some undecomposed cereal material remained in the soil in the triticale treatment at the end of the second sowing of this pot trial.

Full results are included in Appendix 2.6. No interaction between inoculum level and green manure treatments was observed for any of the variables measured.

Table 6.9 Factors showing differences between treatments at harvest of green manures trial 1, second sowing.

Green Manure	Number of tubers per plant	Mean tuber weight, (g)	Electrical conductivity, (µS/cm)
None (control)	2.0 a[1]	7.8 a	78 a
Broad bean	2.2 a	9.9 a	106 b
Triticale	2.8 b	13.4 b	112 b
Standard Error	0.18	0.81	8.6

[1] Figures in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher's Least Significant Difference Test. Each figure is the mean of 6 replicates with 9 plants per replicate.

6.3.2 Second green manures pot trial

6.3.2.1 Green manures dry matter and C:N determinations

In this pot trial dry matter mass applied to each pot was the same for each green manure type at 6.4 gkg^{-1} of soil (Table 6.10). The C:N ratio varied from 41.4 for rye to 29.9 for mustard and 12.9 for lupin.

Table 6.10 Dry matter and C:N determinations for green manures pot trial 2.

	Green manure		
	Lupin	Rye	Mustard
Vegetation analyses			
Vegetation dry matter, (%)	16.9	33.7	19.7
Carbon content, (%).*	44.64	43.89	40.60
Nitrogen content, (%).*	3.47	1.06	1.36
C:N ratio	12.9	41.4	29.9
Green manure contributions to soil			
Vegetation dry matter added to soil (g/kg)	6.4	6.4	6.4
Dry matter per soil surface area in pots (kg/m^2)	0.82	0.82	0.82
Maximum carbon added to soil, (g/kg).	2.86	2.81	2.60
Maximum nitrogen added to soil, (g/kg).	0.22	0.07	0.09

* Percentage of green manure dry weight, determined by Carlo Erba 1108 CHNO-S analyser.

6.3.2.2 First sowing

Scab was not observed in any treatment. However, network russetting was present, being noticeably more prevalent in the rye treatment (Figure 6.11) and did not appear to be affected by inoculum type.

Neither weight nor number of tubers differed between treatments and averaged 16.6 g and 2.7 per plant respectively. Tuber yield data showed no evidence of interaction between green manures and inoculum.

Inoculation with either strain 2/2-5 or 25/2 produced no measurable effect on any of the variables measured.

Mean soil pH was somewhat greater than expected (for a krasnozem soil) at 7.8 and did not differ between treatments. Electrical conductivity was significantly elevated in all three green manure treatments (Table 6.11). Cellulase activity was very significantly increased in green manure treatments ($p<0.001$) and also significantly greater in lupin and rye than in the mustard treatment. Although a similar trend was indicated for FDA hydrolysis activity, treatment differences were not significant. Use of composite samples meant that the effect of any interaction between green manures and inoculum on soil properties could not be determined.

Table 6.11 Factors showing differences between treatments at harvest of green manures trial 2, first sowing.[#]

Green Manure	Electrical conductivity, (μS/cm)	Cellulase activity [§]
None (control)	159 a[1]	13.1 a
Lupin	224 b	29.4 c
Rye	214 b	30.9 c
Mustard	219 b	23.9 b
Standard Error	12.9	1.41

[#] Figures in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher's Least Significant Difference Test. Each figure is the mean of 3 replicates with 3 plants per replicate.

[§] Percent reduction in viscosity of a NaCMC solution.

Full data for Green manures trial 2 – first sowing is included in Appendix 2.7.

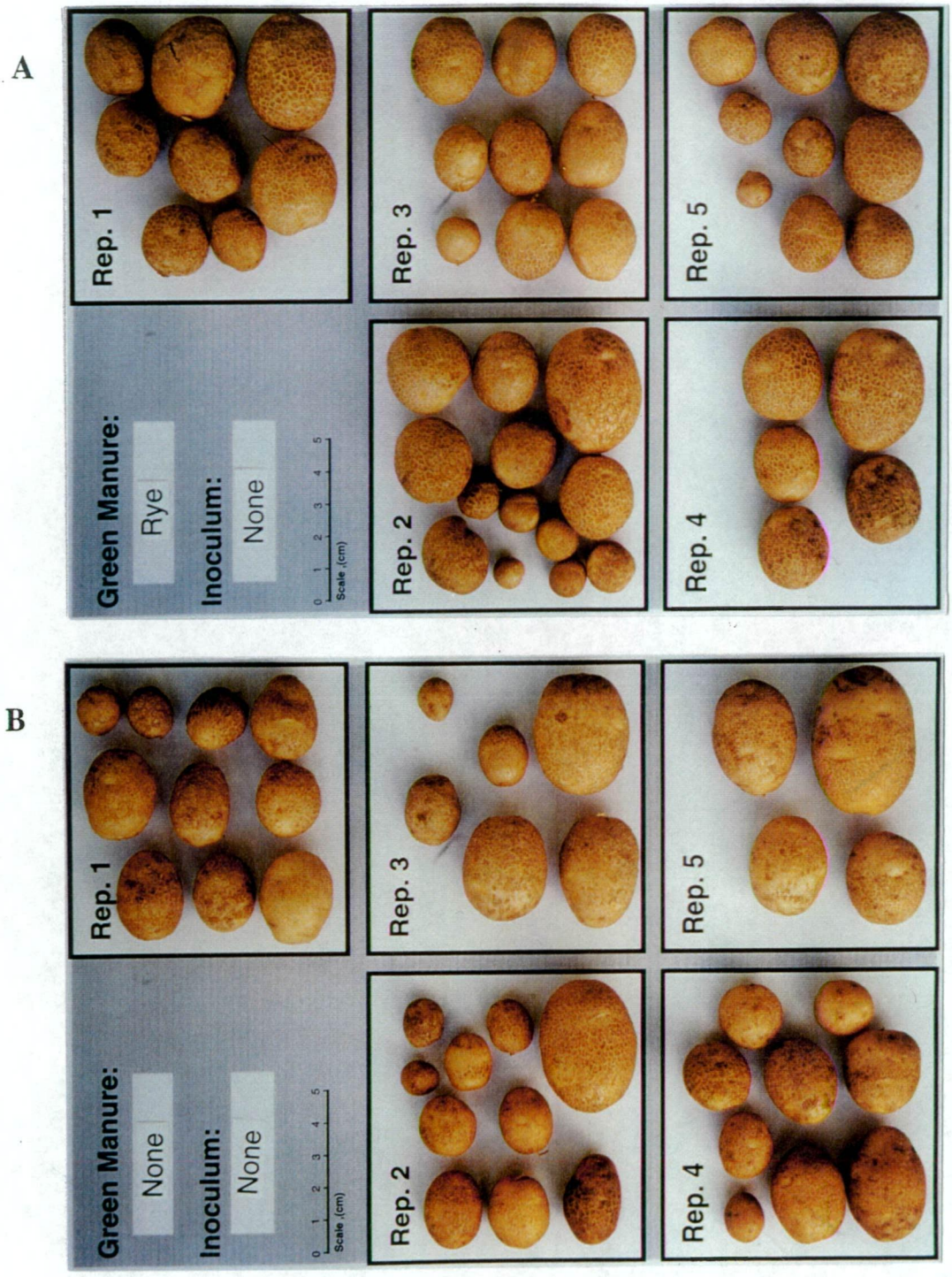


Figure 6.11 Green manures pot trial 2 - first sowing. Surface rusting on tubers (cv= Russet Burbank) from the uninoculated: **A)** rye and; **B)** control (no green manure) treatments. Russetting was most pronounced in the treatments to which rye had been added as a green manure and not appreciably affected by inoculum type.

6.3.2.3 Second sowing

Experimental data for the second sowing of green manures trial 2 is summarised in Appendix 2.8.

Scab was very mild, averaging less than one lesion per plant and not differing between treatments.

There were no effects of inoculum or of interaction between inoculum and green manures on the parameters measured.

Tuber yield was greater in the lupin treatment compared to mustard, with other treatments being intermediate (Table 6.12).

The differences in cellulase activity proved to be very significant ($p= 7.8x10^{-10}$) with all green manures being greater than the control although activity associated with mustard was less than for the other green manures.

Electrical conductivity remained greater in green manure treatments compared to the control.

Table 6.12 Factors showing differences between treatments at harvest of green manures trial 2, second sowing.[#]

Green Manure	Tuber weight per plant, (g)	Electrical conductivity, (µS/cm)	Cellulase activity [§]
None (control)	14.3 abc	151 a[1]	23.0 a
Lupin	17.6 cd	187 b	30.5 c
Rye	15.9 bcd	178 b	31.9 c
Mustard	12.2 ab	175 b	27.7 b
Standard Error	0.86	8.3	0.79

[#] Figures in each column followed by the same letter are not significantly different at $p<0.05$ according to Fisher's Least Significant Difference Test. Each figure is the mean of 15 replicates with 3 plants per replicate.

[§] Percent reduction in viscosity of a NaCMC solution.

6.3.3 Influence of *S. scabies* and *S. violaceusniger* on growth of green manure crops

Neither strain 1/2-3 nor 46/1A-1 were associated with any significant variation in vegetation dry weights of manure crops when evaluated in potting mix (Appendix 2.9).

Actinomycetes plate counts on GAT averaged $1.9x10^5$ cfu g^{-1} of potting mix and did not differ significantly between treatments. On these plates an unknown actinomycete producing yellow spores predominated, greatly outnumbering colonies which resembled the inoculated strains.

Final pH of the potting mix was 7.3 and mean electrical conductivity 160 μScm^{-1}.

6.3.4 Field trials

Climate at both sites during the time of the trials was in a range suitable for potato cropping (Appendix 3.3). Rainfall for much of the period was below average, meaning that greater irrigation was required than usual.

6.3.4.1 Green manure dry matter and C:N determinations

Vegetation C:N ratios were in the range expected for the green manure crops used (Table 6.13). Dry matter added at field sites per unit soil area ranged between 0.22 and 0.47 kgm^{-2}. The dry matter mass added at field sites (in kgm^{-2}) was less than for the pot trials, being between 9% and 29% of that added in pot trial 1 and between 27% and 57% that applied in pot trial 2 (Tables 6.4 and 6.10).

Some undecomposed cereal material remained in the soil in the oat treatment at potato harvest at the Springfield site.

Table 6.13 Carbon, nitrogen and dry matter determinations for green manures at field sites

	Green manure						
	Springfield site				Deloraine site		
	Canola	Oats	Lupin	Ryegrass	Oats	Lupin	Ryegrass
Vegetation analyses							
Vegetation dry matter, (%)	13.6	15.9	16.2	18.5	18.8	21.0	17.5
Carbon content, (%).*	39.76	40.97	40.32	40.43	41.99	39.68	39.58
Nitrogen content, (%).*	2.75	2.10	2.89	1.78	2.37	2.93	2.28
C:N ratio	14.4	19.5	14.0	22.7	17.7	13.5	17.4
Green manure contributions to soil							
Dry matter per unit area, (kg/m^2)	0.26	0.38	0.23	0.23	0.38	0.22	0.47

* Percentage of green manure dry weight, determined by Carlo Erba 1108 CHNO-S analyser.

6.3.4.2 Effects on tuber yield

Green manure treatments had no effect on tuber weights, tuber numbers per plant or on overall tuber yield at either site (Appendix 2.10). Tuber numbers per plant averaged 10.5 at the Springfield site and 10.7 at the Deloraine site. Mean tuber weight was 232 g at Springfield and 353 g at Deloraine. There was significant ($p= 0.046$) interaction between treatments and blocks in mean tuber weights across the Springfield site, showing as a significant difference between sample points in the fallow treatment only but indicating some variability in potato growth conditions.

6.3.4.3 Effects on scab severity

Scab severity, being very mild, was recorded at both sites as numbers of lesions. Typical scab lesions were raised, corky and generally 0.5 to 1.0 cm in diameter (Figure 6.12).

Figure 6.12 A tuber from the Deloraine site showing scab lesions.

The pattern of scab severity differed between sites. At Deloraine, scab (expressed as number of lesions per plant) was significantly greater for the oat treatment than for the other treatments (Table 6.14). In contrast the oat treatment did not differ significantly from the fallow control treatment at the Springfield site. For those treatments replicated across both sites, scab disease incidence was generally lower at Springfield than Deloraine.

Canola (at Springfield, not replicated at Deloraine) was associated with significantly greater ($p \geq 0.01$) scabbing than any other treatment, both in terms of lesions per tuber and lesions per plant.

No significant block effects or interactions between treatments and blocks were observed for scab data.

Table 6.14 Scab severity associated with green manure treatments at field sites #.

	Green Manure					S.E.§
Deloraine Site	Oat	Lupin	Ryegrass	Fallow		
Scab lesions per plant	12.0 a	4.8 b	2.5 b	3.9 b		2.25
Scab lesions per tuber	1.04	0.49	0.23	0.59		0.237
Springfield Site	Oat	Lupin	Ryegrass	Fallow	Canola	
Scab lesions per plant	0.2 b	1.9 b	1.8 b	0.8 b	33.4 a	6.02
Scab lesions per tuber	0.01 b	0.15 b	0.14 b	0.08 b	3.74 a	0.592

Figures in each row followed by the same letter are not significantly different at $p<0.05$ according to Fisher's Least Significant Difference Test. Each figure is the mean of all tubers ≥ 50g from 3 replicate groups of 4 plants.

§ S.E. = Standard error

6.3.4.4 Effects on soil microbial activity, electrical conductivity and pH

Changes observed in levels of FDA hydrolysis, cellulase, soil pH and electrical conductivity for the two trial sites over the period of these trials are plotted in Figure 6.13. These variables generally did not differ significantly (at $p\leq0.05$) between treatments at any sampling date ("NS" shown above the graph). Where differences were observed a $lsd_{(0.05)}$ bar is shown. The corresponding data are also included in Appendix 2.11.

Neither cellulase activity nor soil pH differed between treatments at any of the sampling dates at either site. Soil at the Deloraine site had a higher pH and consistently lower soil enzyme activities than the Springfield site.

Springfield

Treatment differences in soil FDA hydrolysis activity were observed on the last three sampling dates. FDA hydrolysis activity was significantly greater in all green manure treatments relative to fallow at the time of maximum green manure growth, with activity associated with the lupin treatment being greater than all others. At three weeks after potatoes were sown (approximately 2.5 months after ploughing in green manures) FDA hydrolysis activities were greater in the ryegrass

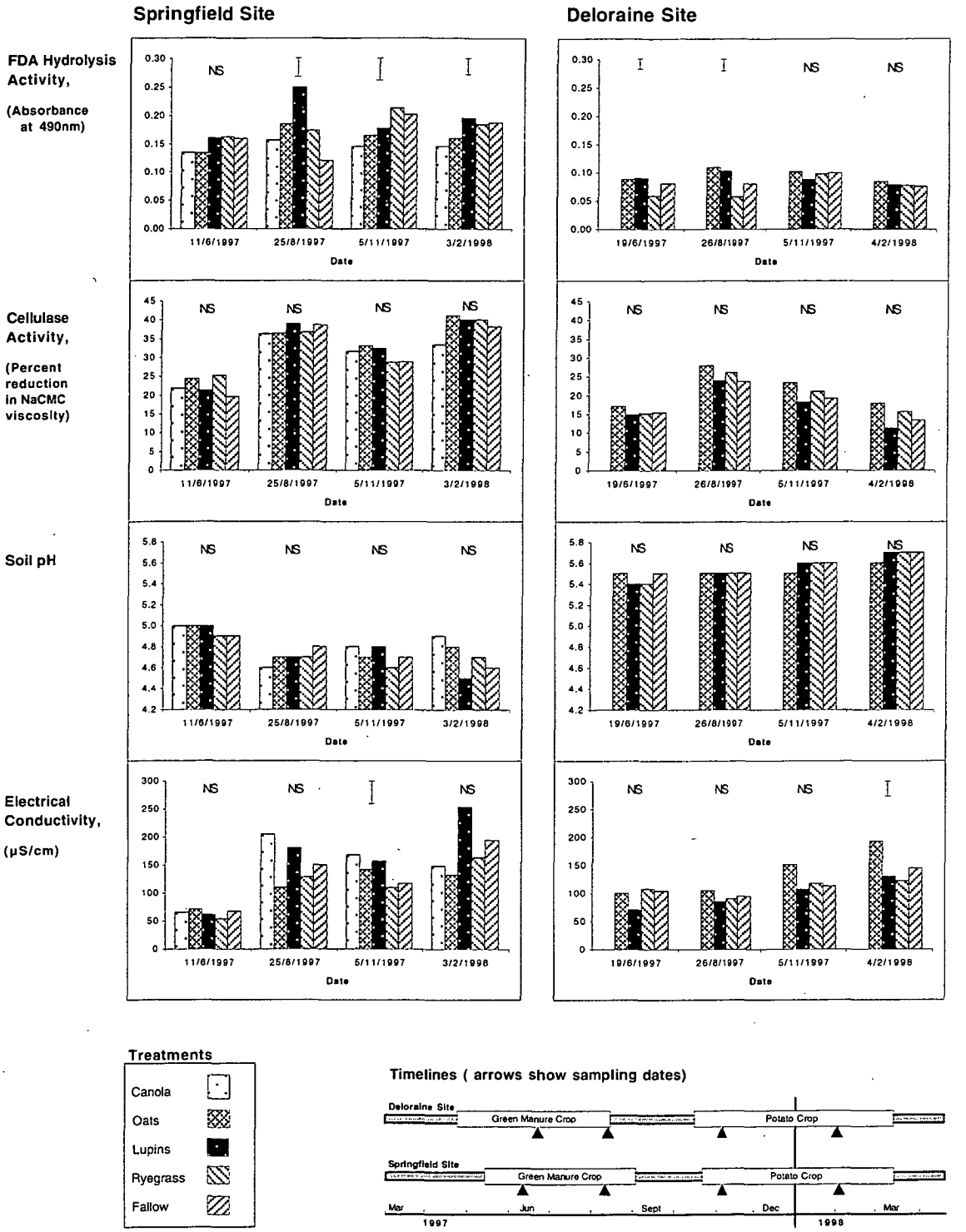


Figure 6.13 Changes in soil FDA hydrolysis, cellulase, pH and electrical conductivity over time in green manure plots at the two field sites. Above columns for each sampling date is included either: a bar showing $LSD_{0.05}$ (where means for that sample date were not different at $p \leq 0.05$) or; NS where not significant. Each column is the mean of three replicates. Data are included in Appendix 2.13. Timelines inset shows relationship between sampling dates and stages of green manure and potato crop growth.

and fallow treatments than in the canola treatment. The pattern was similar at the time of advanced growth of the potato crop with FDA hydrolysis activity in the canola treatment remaining lower than all but the oat treatment. At this time, activities associated with lupin and fallow treatments were also greater than the oat treatment.

Soil electrical conductivity at the Springfield site differed between treatments on only the third sampling date. At this time, conductivity in canola was greater than for fallow or ryegrass and also greater in lupin than for ryegrass. Although mean conductivities were apparently elevated for some treatments at the second and fourth sampling dates, increased variability within treatments meant that these differences were not significant.

Deloraine

Soil FDA hydrolysis activity was lower within ryegrass than other treatments at both sampling times during green manure crop growth phase. At the second of these times, when plants were more mature, activity was also greater with oats and lupin than for fallow. The only other difference at this site was in electrical conductivity, which was greater in the oat treatment than all others at the final sampling.

6.3 Discussion

6.3.1 Effect of green manures on common scab

Of the pot trials, it was only in the first sowing of the first green manures trial that measurable treatment effects on scab severity were found. In this trial, scab was reduced with broad bean but not with triticale. The difference in scab severity was small in absolute terms with average tuber surface area scabbed in the broad bean treatment being 1.47% compared to 2.73% for the control. Nevertheless this result does show that a broad bean green manure can reduce the severity of common scab symptoms in this soil type. Although changes in electrical conductivity, pH, cellulase activity, and exchangeable K and Mg in soil were correlated with green manure addition in the pot trials, these changes were not correlated with scab severity.

Scab incidence at field sites was very mild and may be regarded as insignificant. However, lesion counts per plant did vary enough for differences to be measured. Site to site variation meant that an oat treatment was associated with increased scab at one site but no increase at the other. On balance it may be concluded that the oat manure had no consistent effect on scab severity in these trials. A Brassica green manure (canola) was associated with increased scab at one field site. Although indicating that a canola green manure may promote scab, this result should also be treated with caution as it was observed at one site only and not replicated elsewhere. Further replication over a range of sites would be needed to confirm any scab conduciveness associated with this crop.

Use of green tissue of *Brassica* species as a soil amendment has been shown elsewhere to have suppressive effects on soil organisms, including fungi (Meuhlchen *et al.*, 1990; Papavizas and Lewis, 1971; Ramirez-Villapudua and Munnecke, 1988; Subbarao *et al.*, 1994) and nematodes (Mojtahedy *et al.*, 1993) and on weed species (Boydston and Hang, 1995). This suppression can be attributed to glucosinolate production by the *Brassica* species (Lewis and Papavizas, 1971). Enzymatic hydrolysis of glucosinolates results in the production of a range of compounds including isothiocyanates (Duncan, 1991) which have fungicidal properties.

A possible mechanism by which the canola green manure could have increased scab incidence may have been by alteration in the balance of the soil microbiota brought about by antimicrobial substances produced by the green manure. It is possible that suppression of other competing soil biota may have allowed the common scab pathogen a greater opportunity to colonise potato tubers. FDA hydrolysis activity associated with the canola treatment tended to be lower than other treatments, lending support for the possibility that microbial activity was suppressed in this treatment.

At the Deloraine site, where scab disease was significantly greater in the oat treatment, soil electrical conductivity also tended to be greater in this treatment at both sample times during the potato growth phase and was significantly greater at the last sampling. It is possible therefore that this increased electrical conductivity may be related to the increased disease incidence. No concurrent difference in pH between the oat and other treatments was noted.

In addition to replication over a number of sites, the usefulness of field based green manure experiments might be enhanced by extending them to cover several seasons, such as Weinhold *et al.*, (1964) have done. Changes in soil properties or microbial inoculum levels may not manifest effects on scab disease severity until the following or later seasons.

6.4.2 Effects of antagonist inoculum

With the exception of some apparent effects of strain #23 on tuber yield in the first pot trial, added antagonist inoculum was not associated with any significant changes in the variables measured. The changes in tuber yield associated with strain #23 were not consistent between the two sowings of the same pot trial, with a reduction occurring in the first sowing and an increase in the second sowing. If these were real effects, then the mechanism remains to be determined and may be due to interaction between strain #23 and the potato plants, *S. scabies* or with other soil microbiota. Strain #23 has potent antifungal activity against a range of plant pathogens (C. Wilson, pers. com.). Antagonist strains 2/2-5 and 25/2 were not observed to have any effects on any of the variables measured. It is possible that neither of these two strains were antagonists of *S. scabies* as they had been predominantly selected for antagonism to *S. violaceusniger*.

There can be no certainty that any of the antagonists had grown in the soil as there was no easy way to distinguish them from similar actinomycetes found in soil plated out onto agar media. In order to study the growth of any strain in soil it would be necessary to have a reliable marker so that that strain can be clearly distinguished amongst others.

The concept of applying a microbial antagonist with a green manure is appealing in that potentially the decomposing green manure material can provide a growth substrate for the antagonist. Thus in the process, the need to mass produce a large amount of prepared inoculum can be circumvented. This may work if the antagonist is well adapted to growth on the decomposing organic material. Nevertheless, it is unlikely that any added organism is going to be better adapted to colonising this niche than others that are already present in the soil. A better approach may be to encourage suppressive organisms already present or as Garrett (1955) asserted that soil management, rather than the addition of individual antagonists, was the key to inducing soil suppressiveness.

A streptomycete antagonist had not been applied with the green manures in the field trials as no beneficial effects of doing so had been demonstrated in glasshouse trials. There were a number of other factors taken into consideration in choosing not to apply microbial inoculum to field plots. There was no easy way to show that an antagonist once applied in one place had not been redistributed to other places through soil tillage or water movement (without being confined by a physical barrier such as a pot). It was not known whether any of the antagonists could survive and multiply in the soil. If however an antagonist could survive and multiply in the soil it was not known whether it may have unknown lasting detrimental qualities.

6.4.3 Other effects of green manures

Nitrogen content and vegetation dry weights varied between green manures and between experiments. Nitrogen content of vegetation was greater for legumes than for cereals in the pot trials, a difference that was less pronounced at the field sites. Dry weight of vegetation per kilogram of soil was greatest for triticale (first pot trial), being 1.6 times that of broad bean (applied at equal fresh weight), and 2.1 times that of green manures used in the second pot trial. To get an approximate comparison of quantities used at field sites with pot trials, dry matter

can be calculated as a function of soil surface area (a comparison that does not take into account depth of mixing). Total dry matter applied per unit area was lower at the field sites than for the pot trials. Dry matter had been applied at approximately 6.5 times the field rate in the first pot trial and approximately two to three times the field rate in the second pot trial.

A measure of the rate of decomposition can be inferred from results of the first green manures pot trial. Decomposition of the broad bean green manure occurred at a more rapid rate than triticale, as indicated by changes in soil cellulase activity over the first 70 days after incorporation into the soil (Figure 6.10). Cellulase activity had risen in both green manure treatments by day 30. By day 70 cellulase activity in the broad bean treatment had dropped to a level no different to the control, indicating exhaustion of cellulosic material. Persistence of cellulosic material in the triticale treatment, as shown by the presence of undecomposed material at the end of the experiment, may indicate a slower decomposition rate. This is likely, considering the large volume applied (13.6 g/kg dry matter with C:N ratio of 32.6). It is likely that decomposition of the triticale material was a net drain on soil nitrogen as evident by the slight chlorosis of plants (Figure 6.1). In contrast, the greener vegetation in broad bean treatments indicated an adequate nitrogen supply. There were no apparent differences between treatments in the appearance of potato plants in the second pot trial or in the field trials, indicating adequacy of nitrogen supply in these trials.

As well as affecting plant growth, it is likely that this difference in soil nitrogen balance would have effects on the activity and population balance of soil microbiota. Plate counts revealed no differences between treatments in total numbers of fungi, actinomycetes and other bacteria. Although counts of actinomycetes (+/- melanin) and other bacteria tended to be higher in the broad bean treatments (Appendix 2.5) limited replication meant that no statistically significant difference was found. Both agar media used were probably sub-optimal for counts of absolute numbers of fungi and bacteria in soil. However counts in this case were comparative. Growth of microorganisms on carboxymethylcellulose agar (CMCA) may indicate that this medium has potential for isolation or enumeration of cellulose degraders.

Increased network russetting associated with rye in the second green manures pot trial may be associated with effects of the green

manure on the soil microbial balance. The pattern of lesions, being over the whole tuber and without obvious signs of cell hypertrophy, was not typical of common scab and appeared more characteristic of netted scab (Scholte and Labruyere, 1985). It is possible that this russetting may have been produced as a response of the soil biota to soil conditions, such as nitrogen levels (Bang, 1995).

Green manures were observed to have a number of effects on tuber yield and on the measured soil properties. Greater volumes of green manures applied in the first pot trial may have been responsible for the greater differences in tuber yields, pH and electrical conductivity found with this pot trial. Much lower green manure volumes could have at least partly accounted for the smaller effects found at field sites

Increased tuber yields associated with green manure addition in the first pot trial was recorded in the absence of added fertilisers and may therefore have been a nutritional effect, due to nutrients released from the green manure material. Similar increases in tuber yield were not observed for the second pot trial where green manures were grown *in situ*, with the exception of lupin treatment where a slight increase in yield may be attributable to nitrogen fixation by the lupin plants.

Green manures were associated with a number of effects on soil properties. Soil pH was marginally reduced in green manure treatments in the first pot trial but not measurably affected in the second pot trial or the field experiments.

Green manure treatments were also associated with increased soil electrical conductivity, indicating a corresponding increase in soluble salts in the soil. These soluble salts may have been sourced from the green manures directly or may have been released from soil minerals as a result of interaction with the added organic matter. In the first pot trial, concentration of exchangeable Mg and K were increased in green manure treatments and probably accounted for some of the increase in electrical conductivity. As the green manures used in this pot trial had been obtained from an external source the increases in soluble salts and conductivity could be via either of the two mechanisms noted above.

In the second pot trial the green manure plants had been grown in the same pots in which they were later applied as green manures. There had been no other materials other than water applied to the pots, meaning that observed increases in electrical conductivity must have

been produced as a result of growth or decomposition of the green manure plants.

6.4.4 Effect of *S. scabies* or *S. violaceusniger* on growth of green manure plants

In the experiment in which *S. scabies* or *S. violaceusniger* had been inoculated into potting mix, these strains did not appear to have any effects on growth of green manure plants as there were no measurable differences in vegetation dry weights. This result can, however, not be taken as proof that these strains do not affect plant growth. Indeed, *S. scabies* has been previously shown to colonise plant roots and affect root weights (Hooker 1949; Leiner *et al.*, 1996). Results of studies elsewhere in this thesis also show that both of these species can affect growth of radish seedlings. It is not possible to determine whether the results of this pot trial were due to the inoculated strains as they were not the predominant species isolated from the soil at the end of the experiment.

7 Summary of Findings

This thesis provided some answers to the questions originally posed, while answers to others remained elusive. In addition new observations were made, which could have implications for future research.

Investigations were conducted into streptomycete isolates from potatoes collected from across the Tasmanian potato cropping region, into the scab conduciveness of soils from those regions and into the effects of green manures on common scab. An important limitation, impinging on the outcome of all three research areas, was of low levels of disease observed in pot or field trials.

Physiological and biochemical characteristics of strains isolated from scab lesions of potatoes were determined, enabling taxonomic grouping and identification of 68 of the 94 strains. These strains included *S. scabies, S. violaceusniger, Streptosporangium* spp. and strains tentatively identified as *S. halstedii.* Some of the 26 ungrouped strains resembled *S. scabies.*

Two forms could be discerned amongst strains fitting the description of *S. scabies.* The most abundant among these produced grey-brown spores in tight spirals and could degrade xanthine. Members of this group could grow at pH 4.5 as opposed to pH 5.0 minimum reported for *S. scabies* strains found elsewhere (Lambert and Loria, 1989a). This group included an Australian *S. scabies* reference strain (#32) known to be a pathogen and also known to produce thaxtomin A. A second smaller group of strains resembling *S. scabies* produced grey spores in loose spirals and did not degrade xanthine, a description more characteristic of the type strain.

S. violaceusniger strains resembled *S. scabies* in using all nine ISP sugars and in producing grey spores in spiral chains. Most *S. violaceusniger* strains did not degrade starch, which would appear to not predispose them for pathogenicity to potatoes. However these strains were found to grow vigorously on potato tuber slices.

Three *Streptosporangium* strains were found amongst the tuber isolates. These showed no signs of pathogenicity on potato tuber slices and are probably a saprophytic inhabitant of the tuber surface.

Twenty nine strains closely fitted the probabilistic description of *Streptomyces halstedii* (Williams *et al.*, 1983a) although differing in production of a diffusible pigment not normally found with *S. halstedii*. As tested strains showed no clear signs of pathogenicity it is likely that these *S. halstedii*-like strains are also saprophytic.

Pathogenicity of strains was evaluated in four ways. All 94 strains were first screened using a potato disk (slice) assay. In this assay all *S. violaceusniger* strains, *S. scabies* strain #32 and five *S. scabies*-like ungrouped strains showed clear signs of pathogenicity. A smaller selection of strains were re-evaluated in radish seedling and minituber assays. Media from OMB cultures of *S. scabies* and *S. violaceusniger* strains inhibited radish seedling growth, while seedlings were killed by *S. violaceusniger* strains 54/3 and 75/1-1. Tuber surface necrosis and necrosis of green leafy tissue occurred within 24 hours of dipping potato minitubers in media from OMB cultures of *S. violaceusniger*. Three *S. scabies* strains (including #32) also produced slight darkening of lenticels, indicating some necrotising potential. As #32 is a known pathogen this provides evidence that at least strains 1/2-3 and 12/1A may be pathogenic amongst *S. scabies* strains. These three strains had been amongst the most inhibitory of *S. scabies* strains in the radish seedling assay and showed a similar pattern of inhibition in that assay. Culture extracts were assayed of by TLC and mass spectrometry and as a result *S. violaceusniger* strains were found to produce nigericin while strains 54/3 and 75/1-1 also produced geldanamycin, probably accounting for their virulence in pathogenicity assays. Production of thaxtomin A by strain #32 was also confirmed by TLC.

Pathogenicity was not conclusively demonstrated on potato plants growing in sand, for any of four *S. violaceusniger* or *S. scabies* strains including a known positive control (*S. scabies* strain #32), as lesions were mild and atypical and Koch's postulates were not fulfilled.

There was no correlation between source lesion type and pathogenicity results for any of the taxonomic groups.

This investigation gives the first report of scab-like necrosis of potato minitubers by culture media from *S. violaceusniger* strains. The

ecological significance of *S. violaceusniger* on potato remains to be determined. It is possible that this species may be responsible for a form of scab on potatoes. If not, then *S. violaceusniger* may have potential as an antagonist of *S. scabies*.

Scab severity associated with 36 soils from the Tasmanian potato growing region was compared in a glasshouse trial. This study gave indications that krasnozem soils are less conducive to common scab at low pH or at low combined levels of the exchangeable cations Ca^{2+}, Mg^{2+} and K^{+}. Threshold levels were found, for pH and exchangeable cations, below which scab disease was less likely. The pH threshold of around 5.2 units is in accordance with similar findings from elsewhere and indicates the robustness of this threshold over a range of soil types. A strong correlation between soil pH and exchangeable cations, particularly calcium, was also found.

Results of green manures experiments were confounded by low levels of scab in control treatments. In no pot trial was disease significantly increased by green manures. A broad-bean green manure was associated with a slight but significant reduction in scab severity in one pot trial. In field trials the use of lupin or ryegrass as a green manure had no effect on scab severity. An oat manure gave inconsistent results with a marginal increase in scab at one site and no significant change at the other. On the site where disease was increased in association with the oat manure there was a concurrent increase in soil electrical conductivity. Disease was also increased with a canola green manure, evaluated at one site only, and associated with reduced FDA hydrolysis activity (indicating reduced soil microbial activity). Further experimentation would be required to clarify scab conduciveness of any crop, or of continued effects over additional seasons.

In glasshouse evaluation of green manures these were associated with increased cellulase activity and electrical conductivity of soil in the absence of added fertiliser plus, in some trials, increased tuber yields. At the field sites, green manures did not affect tuber yield, cellulase activity or pH.

Three potential scab antagonists were evaluated in pot trials in combination with green manures. *S. scabies* strain #23 has previously been shown elsewhere to have scab antagonist potential while strains 2/2-5 (tentative *S. halstedii*) and 25/2 (tentative *S. scabies*) had been selected from amongst strains evaluated in this project. None of these

strains showed significant evidence for suppression of scab in pot trials when applied as a spore suspension alone or in combination with green manures.

A new culture medium containing carboxymethyl cellulose as a carbon source (CMCA) was devised. This medium may have potential for enumeration of cellulose degraders from soil.

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Appendix 1 Media and Reagents

1.1 Culture Media

All media were autoclaved for 15 minutes at 121°C unless otherwise specified.

1.1.1 **Glycerol Asparagine Agar** (ISP Medium 5; Pridham and Lyons, 1961)

Glycerol	10.0 g
L- Asparagine (Sigma)	1.0 g
K_2HPO_4	1.0 g
Agar	16.0 g
Trace Salts Solution (see Appendix 1.13)	1.0 ml
Distilled water	1000 ml

Trace salts solution added to medium after it had been autoclaved and cooled to about 50°C.

1.1.2 **Glycerol Asparagine Tyrosine Agar** (GAT)

As for ISP Medium 5, with the addition of 2.0 g tyrosine per litre.

1.1.3 **Yeast Extract Malt Extract Agar** (YME; ISP Medium 2; Pridham *et al.* 1957)

Malt extract (Oxoid)	10.0 g
Yeast extract (Oxoid)	4.0 g
Glucose	4.0 g
Agar	16.0 g
Distilled water	1000 ml

Adjust pH to 7.3 ± 0.2 (at 25°C) before autoclaving.

1.1.4 **Peptone Yeast Extract Iron Agar** (PYI; ISP Medium 6; Shirling and Gottlieb, 1966)

Peptone (Oxoid)	15.0 g
Proteose peptone (Oxoid)	5.0 g
K_2HPO_4	1.0 g
Yeast Extract (Oxoid)	1.0 g
Ferric ammonium citrate	0.5 g
$NA_2S_2O_3$	0.08 g
Agar	15.0 g
Distilled water	1000 ml

1.1.5 **Tyrosine Agar** (Shirling and Gottlieb, 1966)

Glycerol	15.0 g
L-Tyrosine (Sigma)	0.5 g
L-Asparagine (Sigma)	0.5 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.5 g
NaCl	0.5 g
$FeSO_4.7H_2O$	0.01 g
Agar	16.0 g
Trace salts solution (see Appendix 1.13)	1.0 ml
Distilled water	1000 ml

Adjust pH to 7.2 –7.4

1.1.6 **Carbon Source Utilisation Medium** (Lambert and Loria, 1989a)

Basal medium	
$(NH_4)_2SO_4$	2.64 g
KH_2PO_4	2.38 g
K_2HPO_4	4.31 g
$MgSO_4.7H_2O$	1.00 g
$CuSO_4.5H_2O$	6.4 mg
$FeSO_4.7H_2O$	1.1 mg
$MnCl_2.4H_2O$	7.9 mg
$ZnSO_4.7H_2O$	1.5 mg
Agar	15 g
Distilled water	1000 ml

Medium adjusted to pH 6.9. Carbon sources filter sterilised and aseptically added to the basal medium (after it has been autoclaved and cooled to about 50°C) to give a concentration of 1.0% (w/v).

1.1.7 **Nitrogen Source Utilisation Medium** (Lambert and Loria, 1989a)

Basal Medium

Glucose	10.0 g
$MgSO_4.7H_2O$	5.0 g
NaCl	5.0 g
K_2HPO_4	1.0 g
$FeSO_4.7H_2O$	10 mg
Agar	15 g
Distilled water	1000 ml

Medium adjusted to pH 7.4. Nitrogen sources filter sterilised and aseptically added to the basal medium (after it has been autoclaved and cooled to about 50°C) to give a concentration of 1.0 gl^{-1}.

1.1.8 **Modified Bennett Agar** (Lambert and Loria, 1989a)

Beef extract	1.0 g
Yeast extract	1.0 g
Tryptone	2.0 g
Glycerol	10.0 g
Agar	15.0 g
Distilled water	1000 ml

1.1.9 **Hankin Pectin Medium** (Hankin *et al.* 1971, as cited by Williams *et al.* 1983b)

Part A

Na_2HPO_4	6.0 g
Distilled water	200 ml

Part B

Pectin (Sigma)	5.0 g
Distilled water	200 ml

Part C

$(NH_4)_2SO_4$	2.0 g
$FeSO_4.7H_2O$	0.001 g
Yeast Extract (Oxoid)	1.0 g
$MgSO_4.7H_2O$	0.2 g
Agar	10.0 g
Distilled Water	600 ml

Autoclave each part seperately before combining. Final pH should be 7.4.

1.1.10 Hydrolysis of Arbutin (Kutzner, 1976, as cited in Williams *et al.*, 1983b)

Amount (per litre) medium	Test medium	Control
Yeast extract	3.0 g	3.0 g
Arbutin	1.0 g	0.0 g
NH_4^+-Fe^{3+}-citrate	0.5 g	0.5 g
Agar	3.0 g	3.0 g

Adjust pH to 7.2. Each strain is tested both media (i.e. +/- arbutin).

1.1.11 Medium for assessing pH sensitivity (Lambert and Loria, 1989a)

Basal medium

Glucose	10.0 g
L-Asparagine	0.5 g
Agar	15.0 g
Distilled water	1000 ml

Media contained 0.040 M phosphate added as orthophosphoric acid, monopotassium and dipotassium salts to give a pH range of 3.5 to 6.5 in 0.5 unit increments.

1.1.12 **Medium to assess growth with NaCl** (Kutzner, 1981)

Basal medium (per litre):

Glucose	4.0 g
Yeast extract	4.0 g
Malt extract	10.0 g
Agar	12.0 g
Distilled water	to 1000 ml

Add NaCl to media at 5, 6 and 7% (w/v). Adjust pH to 7.2.

1.1.13 **Trace Salts Solution** (Pridham and Gottlieb, 1966)

$CuSO_4.5H_2O$	0.64 g
$FeSO_4.7H_2O$	0.11 g
$MnCl_2.4H_2O$	0.79 g
$ZnSO_4.7H_2O$	0.15 g
Distilled water	to 100 ml

Filter sterilise

1.1.14 **Oatmeal Agar** (ISP Medium 3; Shirling and Gottlieb, 1966)

Oatmeal (Quaker Oats)	20.0 g
Trace salts solution (see Appendix 1.13)	1.0 ml
Agar	16.0 g
Distilled water	1000 ml

Boil oatmeal for 15 min in 800 ml water. Filter through cheesecloth then add agar and water to bring to volume. Put into flasks and autoclave. Trace salts solution added to medium after it had been autoclaved and cooled to about 50°C.

1.1.15 Oatmeal Broth

As for Oatmeal Agar with agar omitted.

1.1.16 Sucrose Asparagine Yeast Extract (SAY) Medium

Sucrose	20.0 g
L-Aspagagine (Sigma)	1.2 g
K_2HPO_4	0.6 g
Yeast extract (Oxoid)	10.0 g
Distilled water	1000 ml

1.2 Reagents

1.2.1 Phosphate buffer for esterase assay

K_2HPO_4	8.81 g
KH_2PO_4	1.27 g
Distilled water to 1000 ml	

Adjust, if necessary, to pH 7.6 before making up to volume.

1.2.2 Buffered CMC solution for cellulase assay

Na Carboxymethyl cellulose (BDH)	4.0 g
Citric acid	4.15 g
Na_2HPO_4	8.10 g
Distilled water to 1000ml	

Adjust, if necessary, to pH 5.5 prior to making up to volume.

Appendix 2.1 - Strain Characteristics

Characteristic	Strain	1/1	1/2-3	1/2-4	2/1-1	2/1-3	2/1-4	2/1-5	2/2-2	2/2-3	2/2-4	2/2-5	5/1	6/1B	8/2	9/1	12/1A	12/2A-1	12/2B	13/1B
	Taxonomic group	*Streptomyces scabies*	*S. scabies*	Group 5	Group 5	Group 5	Group 1	Group 1	Group 5	*S. scabies*	Group 1	Group 1	Group 5	Group 5	*S. scabies*	*S. scabies*	*S. scabies*	*S. scabies*	*S. scabies*	*S. scabies*
Spore colour on YME §		GB	GB	G	G	G	G	WY	G	GB	G	G	G	G	GB	GB	GB	GB	GB	GB
Reverse colour §		B	B	OB	RB	B	B	S	B	B	B	G	B	B	B	B	B	B	B	B
Lysis of spore-bearing hyphae ¶		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Spore chain morphology ¥		ts	ts	fl	ls	ls	fl	fl	ls	ts	fl	fl	ls	ls	ts	ts	ts	ts	ts	ts
Melanin on tyrosine agar		+	+	—	+	+	—	—	—	+	—	—	+	+	+	+	+	+	+	+
Melanin on PYI		+	+	—	+	+	—	—	—	+	—	—	+	+	+	+	+	+	+	+
Diffusible pigment on GAT §		—	—	Y	—	—	—	—	—	—	Y	Y	—	—	—	—	—	—	—	—
Carbon usage																				
L-Arabinose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Glucose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol		+	+	+	+	—	+	+	—	+	+	+	—	—	+	+	+	+	+	+
Raffinose		+	+	+	+	+	+	—	+	—	—	—	+	+	+	+	+	+	+	+
Rhamnose		+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose		+	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+
D-Xylose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
meso-Inositol		+	+	+	+	+	—	—	+	+	—	—	+	+	+	+	+	+	+	+
Nitrogen usage																				
L-Hydroxyproline		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Methionine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Degradation of:																				
Arbutin £		a	a	+	a	+	+	+	a	a	+	+	+	+	a	a	a	a	a	a
Polygalacturonate		+	+	—	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+
Xanthine		+	+	+	—	—	+	+	+	+	+	+	—	—	+	+	+	+	+	+
Xylan		+	+	—	+	+	+	+	—	—	+	+	—	+	+	+	—	—	+	+
Starch		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Minimum Growth pH		4.5	4.5	4.5	4.5	5.0	5.5	5.5	4.5	4.0	5.5	5.5	5.5	4.5	4.0	4.5	4.5	4.5	4.5	4.5
Growth with:																				
5%NaCl		+	+	+	—	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+
6%NaCl		—	+	+	—	—	+	+	—	+	+	+	—	—	+	+	+	+	—	—
7%NaCl		—	—	+	—	—	+	+	—	—	+	+	—	—	+	—	—	+	—	—
Tellurite (10µg/ml)		—	+	+	—	+	+	+	—	—	+	+	—	+	+	+	+	+	—	+
Tellurite (100µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Thallium (10µg/ml)		—	+	—	—	—	+	+	—	—	—	+	—	—	+	—	+	+	—	+
Thallium (100µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Crystal Violet (0.5µg/ml)		+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+
Phenol (0.1%)		+	+	+	—	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillin (10 IU/ml)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oleandomycin (25µg/ml)		+	+	+	—	—	+	+	+	+	+	+	—	—	—	—	+	+	+	+
Oleandomycin (100µg/ml)		+	+	+	—	—	+	+	+	+	+	+	—	—	—	—	+	+	+	+
Streptomycin (20µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

NOTES

§ First letter denotes main colour and second letter the predominant hue: G, Grey;B, Brown; W, White;Y, Yellow; P, Pink; S, "Straw coloured";O, Orange.

¶ +, positive; —, negative; nd, not determined.

¥ Spore chain morphology: ts, tight spirals; ls, loose spirals; fl, flexuous; gl, globular.

£ Arbutin degradation: a, not determined (colour masked by pigment).

Characteristic	Strain	14/1A	15/1B	15/2A	15/3A	16/2	16/2-2	18/2B	19/1A-1	19/1A-2	19/1B	20/1A	20/1B	25/2	41/2	42/1-2	42/2-1	43/1-1	43/1-2	43/1-3
	Taxonomic group	*S. scabies*	*S. scabies*	*S. scabies*	*S. scabies*	*S. scabies*	*S. scabies*	*S. scabies*	Group 5	*S. scabies*	*S. scabies*	Group 5	Group 5	Group 5	Group 5	Group 1	Group 1	Group 5	*Streptomyces violaceusniger*	Group 1
Spore colour on YME §		GB	GB	GB	GB	GB	GB	GB	G	GB	GB	G	WG	WG	G	WY	G	G	G	G
Reverse colour §		B	B	B	B	B	B	B	B	B	B	S	B	B	YB	S	B	B	S	B
Lysis of spore-bearing hyphae ¶		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—
Spore chain morphology ¥		ts	ts	ts	ts	ts	ts	ts	ls	ts	ts	ts	ls	fl	ts	fl	fl	fl	ts	fl
Melanin on tyrosine agar		+	+	+	+	+	+	+	—	+	+	—	+	+	+	—	—	—	—	—
Melanin on PYI		+	+	+	+	+	+	+	—	+	+	—	+	+	+	—	—	—	—	—
Diffusible pigment on GAT §		—	—	—	—	—	—	—	nd	—	—	—	—	—	—	—	—	—	PO	Y
Carbon usage																				
L-Arabinose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Glucose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+
Raffinose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+	+	—
Rhamnose		+	+	+	+	+	+	+	+	+	+	—	+	+	+	—	+	+	+	—
Sucrose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Xylose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
meso-Inositol		+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+	+	—
Nitrogen usage																				
L-Hydroxyproline		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Methionine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Degradation of:																				
Arbutin £		a	a	a	a	a	a	a	nd	+	a	a	a	a	a	+	+	a	+	+
Polygalacturonate		+	+	+	+	+	+	+	+	+	+	—	+	+	+	—	+	+	+	+
Xanthine		+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	—	—	+
Xylan		—	+	—	+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+
Starch		+	+	+	+	+	+	+	nd	+	+	+	+	+	+	+	+	+	—	+
Minimum Growth pH		4.5	4.5	4.5	4.5	4.0	4.0	4.5	4.5	4.5	4.5	6.0	4.5	4.5	5.5	5.5	5.5	4.5	5.5	6.0
Growth with:																				
5%NaCl		+	+	+	+	+	+	+	—	+	+	+	—	—	—	+	+	+	+	+
6%NaCl		+	+	+	—	—	—	—	—	—	—	+	—	—	—	+	+	—	—	+
7%NaCl		—	—	—	—	—	—	—	—	—	—	+	—	—	—	+	+	—	—	+
Tellurite (10µg/ml)		+	+	+	+	+	+	+	—	+	+	—	—	—	+	+	+	+	—	+
Tellurite (100µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Thallium (10µg/ml)		+	—	—	+	—	—	+	—	+	+	+	—	—	—	+	—	—	—	+
Thallium (100µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Crystal Violet (0.5µg/ml)		+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—	+	+	+
Phenol (0.1%)		+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	—	+
Penicillin (10 IU/ml)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oleandomycin (25µg/ml)		+	+	+	+	—	—	—	+	+	+	+	—	—	+	+	+	—	+	+
Oleandomycin (100µg/ml)		+	—	—	+	—	—	—	—	—	+	+	—	—	+	+	+	—	+	+
Streptomycin (20µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Characteristic	Strain	44/1A-1	46/1A-1	46/1B-1	46/2B	48/2	49/1	51/1	51/2-1	52/2	53/2	54/2	54/3	55/2	56/2	57/1-2	57/1	59/1	62/1	62/1-2
	Taxonomic group	Group 1	*S. violaceusniger*	Group 1	*S. violaceusniger*	*S. violaceusniger*	*S. violaceusniger*	Group 5	Group 1	Group 1	Group 5	*S. violaceusniger*	*S. violaceusniger*	*S. scabies*	*S. scabies*	*S. scabies*	Group 1	Group 5	*S. violaceusniger*	*S. violaceusniger*
Spore colour on YME §		G	G	G	G	G	G	G	G	G	G	G	G	G	GB	G	G	G	G	G
Reverse colour §		B	S	G	S	S	S	G	B	G	YB	S	S	G	B	B	G	B	S	S
Lysis of spore-bearing hyphae ¶		—	+	—	+	+	+	—	—	—	—	+	+	—	—	—	—	—	+	+
Spore chain morphology ¥		fl	ts	fl	ts	ts	ts	fl	fl	fl	ls	ts	ts	ls	ts	ls	fl	ls	ts	ts
Melanin on tyrosine agar		—	—	—	—	—	—	—	—	—	+	—	—	+	+	+	—	+	—	—
Melanin on PYI		—	—	—	—	—	—	—	—	—	+	—	—	+	+	+	—	+	—	—
Diffusible pigment on GAT §		—	PO	Y	PO	PO	PO	—	Y	—	—	PO	PO	—	—	—	Y	—	PO	PO
Carbon usage																				
L-Arabinose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose		+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+
D-Glucose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol		+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	—	+	+	+
Raffinose		—	+	—	+	+	+	—	—	—	+	+	+	+	+	+	—	+	+	+
Rhamnose		+	+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+
Sucrose		+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	+	+	+
D-Xylose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
meso-Inositol		—	+	—	+	+	+	—	—	—	+	+	+	+	+	+	—	+	+	+
Nitrogen usage																				
L-Hydroxyproline		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Methionine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Degradation of:																				
Arbutin £		+	+	+	+	+	+	+	+	+	+	+	+	a	+	a	+	+	+	+
Polygalacturonate		+	+		+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+
Xanthine		+	—	+	—	—	—	—	+	+	—	—	+	+	+	+	+	—	—	—
Xylan		+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+	+	+	+
Starch		+	—	+	—	—	—	—	+	+	+	+	+	+	+	+	+	+	—	—
Minimum Growth pH		6.0	5.5	5.5	5.0	5.0	5.5	6.0	6.0	5.5	6.0	5.5	5.0	4.5	4.5	4.5	5.5	4.5	5.5	5.5
Growth with:																				
5%NaCl		+	+	+	—	—	+	—	+	+	—	—	+	+	+	+	+	+	+	+
6%NaCl		+	—	+	—	—	—	—	+	+	—	—	+	+	—	+	+	+	+	+
7%NaCl		+	—	+	—	—	—	—	+	+	—	—	—	—	—	+	+	+	—	—
Tellurite (10µg/ml)		+	—	+	—	+	—	+	+	+	+	+	—	+	—	+	+	—	—	+
Tellurite (100µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Thallium (10µg/ml)		—	—	—	—	—	—	—	+	+	—	+	—	—	—	+	+	—	—	—
Thallium (100µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Crystal Violet (0.5µg/ml)		+	—	+	—	+	+	+	+	+	+	+	+	+	—	+	+	—	+	+
Phenol (0.1%)		+	—	+	—	—	—	+	+	+	+	—	—	+	+	+	+	+	+	+
Penicillin (10 IU/ml)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oleandomycin (25µg/ml)		—	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	—	+	+
Oleandomycin (100µg/ml)		—	+	+	—	+	+	—	+	—	—	+	—	—	—	+	+	—	+	+
Streptomycin (20µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—

Characteristic	Strain	63/1	64/1	68/1	72/1-1	72/1-2	72/1-3	73/1-1	73/1-2	73/1-4	73/1-5	75/1-1	76/1-1	76/1-2	77/1	77/3	79/2-1	79/2-3	80/2-1	85/1
	Taxonomic group	Group 1	Group 5	*Streptosporangium* sp.	Group 5	*Streptosporangium* sp.	Group 1	Group 1	Group 5	Group 1	Group 5	*S. violaceusniger*	Group 1	Group 1	Group 1	Group 5	Group 5	Group 5	Group 5	Group 5
Spore colour on YME §		G	G	YP	G	P	G	G	G	G	G	B	G	G	G	GB	G	G	G	G
Reverse colour §		YG	B	YB	B	PB	B	G	S	G	B	S	G	G	S	B	S	PB	PB	S
Lysis of spore-bearing hyphae ¶		—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—
Spore chain morphology ¥		fl	ls	gl	fl	gl	fl	fl	ls	fl	ls	ts	fl	fl	fl	ts	ls	ls	ls	ls
Melanin on tyrosine agar		—	+	—	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—
Melanin on PYI		—	+	—	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—
Diffusible pigment on GAT §		—	—	nd	—	nd	Y	Y	—	Y	—	PO	—	—	—	nd	nd	Y	Y	Y
Carbon usage																				
L-Arabinose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose		+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	—	+	+	+
D-Glucose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol		+	+	—	—	—	+	+	+	—	+	+	+	+	+	—	+	+	+	+
Raffinose		—	+	—	+	—	+	—	+	—	+	+	+	—	—	+	—	+	—	—
Rhamnose		+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose		—	+	+	+	—	+	+	+	—	+	+	+	+	—	+	+	+	+	+
D-Xylose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
meso-Inositol		—	+	—	+	—	—	—	+	—	+	+	—	—	—	—	+	+	+	+
Nitrogen usage																				
L-Hydroxyproline		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Methionine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Degradation of:																				
Arbutin £		+	a	+	+	+	+	+	+	+	a	+	+	+	+	a	+	+	+	+
Polygalacturonate		+	+	—	+	—	+	+	+	+	+	+	+	+	+			—	—	+
Xanthine		+	—	—	—	—	+	+	—	+	—	+	+	+	+	+	—	—	—	—
Xylan		+	+	—	+	—	+	+	—	+	+	+	+	+	+	+	+	+	+	+
Starch		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Minimum Growth pH		5.5	5.5	6.5	4.5	6.5	6.0	5.5	4.5	5.5	5.5	5.5	6.0	5.5	6.5	5.5	4.5	5.0	5.0	6.0
Growth with:																				
5%NaCl		+	—	—	—	—	+	+	—	+	+	+	+	+	+	—	—	+	+	+
6%NaCl		+	—	—	—	—	+	+	—	+	—	+	+	+	+	—	—	+	+	+
7%NaCl		+	—	—	—	—	+	+	—	+	—	—	+	+	—	—	—	+	+	+
Tellurite (10µg/ml)		+	+	+	—	+	+	+	+	+	—	+	+	+	+	+	—	+	+	+
Tellurite (100µg/ml)		—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Thallium (10µg/ml)		—	—	+	—	—	+	+	—	+	—	—	—	—	—	+	—	—	—	—
Thallium (100µg/ml)		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Crystal Violet (0.5µg/ml)		+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenol (0.1%)		+	+	+	+	+	+	+	+	+	—	—	+	+	+	—	—	+	+	+
Penicillin (10 IU/ml)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oleandomycin (25µg/ml)		+	+	+	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—	+
Oleandomycin (100µg/ml)		+	+	+	—	+	+	+	—	+	+	—	—	+	—	—	—	—	—	+
Streptomycin (20µg/ml)		—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Characteristic	Strain	86/1	88/1	93/2	94/1-1	94/1-2	95/1	95/2-1	95/2-2	98/1	105/1-1	105/1-2	105/2	106/1	108/2	#23	#25	#32	95-13-1A
	Taxonomic group	Group 5	Group 1	Group 1	*S. scabies*	*S. violaceusniger*	Group 1	*Streptosporangium* sp.	Group 5	Group 1	Group 1	Group 1	Group 1	Group 1	Group 1	*S. scabies*	Group 1	*S. scabies*	*S. scabies*
Spore colour on YME §		G	WY	G	GB	G	G	YP	GB	G	WG	G	G	G	G	GB	G	GB	GB
Reverse colour §		YB	YB	B	B	S	B	YB	B	G	YB	B	B	B	G	B	S	B	B
Lysis of spore-bearing hyphae ¶		—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Spore chain morphology ¥		fl	fl	fl	ts	ts	fl	gl	ls	fl	fl	fl	fl	fl	fl	ts	ts	ts	ts
Melanin on tyrosine agar		—	—	—	+	—	—	—	+	—	—	—	—	—	—	+	—	+	+
Melanin on PYI		—	—	—	+	—	—	—	+	—	—	—	—	—	—	+	—	+	+
Diffusible pigment on GAT §		Y	—	Y	—	RO	Y	—	—	—	Y	Y	Y	Y	Y	—	—	—	—
Carbon usage																			
L-Arabinose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose		+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+
D-Glucose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol		+	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+
Raffinose		+	—	—	+	+	+	—	+	+	—	—	—	—	—	+	+	+	+
Rhamnose		+	—	—	+	+	+	+	+	+	—	—	+	—	+	+	+	+	+
Sucrose		+	+	+	+	+	+	+	+	+	+	+	—	+	—	+	+	+	+
D-Xylose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
meso-Inositol		+	—	—	+	+	—	—	+	—	—	—	—	—	—	+	—	+	+
Nitrogen usage																			
L-Hydroxyproline		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Methionine		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Degradation of:																			
Arbutin £		+	+	+	a	+	+	+	a	+	+	+	+	+	+	a	+	a	a
Polygalacturonate		—	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+
Xanthine		—	+	+	+	—	+	—	—	+	+	+	+	+	+	+	+	+	+
Xylan		—	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+
Starch		+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Minimum Growth pH		4.5	6.0	6.0	4.5	5.5	5.5	6.5	5.5	5.5	6.0	6.0	6.0	6.0	6.0	4.0	5.5	4.5	4.5
Growth with:																			
5%NaCl		—	+	+	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+
6%NaCl		—	+	+	+	—	+	—	—	+	+	+	+	+	+	+	+	+	—
7%NaCl		—	+	+	+	—	+	—	—	—	+	+	+	+	+	+	+	+	—
Tellurite (10µg/ml)		—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
Tellurite (100µg/ml)		—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Thallium (10µg/ml)		—	+	+	+	—	—	+	—	+	—	+	—	—	+	+	+	+	—
Thallium (100µg/ml)		—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Crystal Violet (0.5µg/ml)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenol (0.1%)		+	+	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+
Penicillin (10 IU/ml)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oleandomycin (25µg/ml)		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—
Oleandomycin (100µg/ml)		—	+	+	+	+	+	+	+	—	+	+	+	—	+	+	+	+	—
Streptomycin (20µg/ml)		—	+	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—

Appendix 2.2

Attributes used in numerical taxonomy, sorted in order of Cramer value[#].

Attribute	Data type	States	Cramer value
Hygroscopic cultures	Binary	0 = absent, 1 = present	1.0000
Growth with meso-inositol	Binary	0 = absent, 1 = present	0.9565
Morphology of spore bearing structures	Multi-state	1 = tight spiral chains 2 = loose spiral chains 3 = flexuous chains 4 = globular sporangia	0.8702
Melanin in tyrosine agar	Binary	0 = absent, 1 = present	0.8443
Minimum Growth pH	Numeric	4.0, 4.5, 5.0, 5.5, 6.0 or 6.5	0.8417
Diffusible pigment at 14 days on GAT	Multi-state	0= none, 1= yellow, 2= pale orange	0.7993
Degradation of xanthine	Binary	0 = absent, 1 = present	0.7965
Degradation of starch	Binary	0 = absent, 1 = present	0.7833
Growth with raffinose	Binary	0 = absent, 1 = present	0.7496
Growth with NaCl	Numeric	0= No growth at 5% 1= Growth at 5% 2= Growth at 6% 3= Growth at 7%	0.7337
Growth with 20µg/ml streptomycin	Binary	0 = absent, 1 = present	0.7327
Growth with 0.1% phenol	Binary	0 = absent, 1 = present	0.6879
Spore colour on YME	Multi-state	1 = grey with brown hue 2 = grey 3 = white with yellow hue 4 = white with grey hue 5 = orange	0.6845
Growth with D-mannitol	Binary	0 = absent, 1 = present	0.6233
Growth with D-fructose	Binary	0 = absent, 1 = present	0.5864
Degradation of Polygalacturonate	Binary	0 = absent, 1 = present	0.5576
Growth with tellurite	Numeric	0= No growth at 10µg/ml 1= Growth at 10µg/ml 2= Growth at 100µg/ml	0.5088
Growth with oleandomycin	Numeric	0= No growth at 25µg/ml 1= Growth at 25µg/ml 2= Growth at 100µg/ml	0.5067
Growth with thallium	Numeric	0=No growth at 10µg/ml 1= Growth at 10µg/ml 2= Growth at 100µg/ml	0.4966
Degradation of xylan	Binary	0 = absent, 1 = present	0.4856
Growth with rhamnose	Binary	0 = absent, 1 = present	0.4759
Growth with sucrose	Binary	0 = absent, 1 = present	0.2920
Growth with 0.5µg/ml crystal violet	Binary	0 = absent, 1 = present	0.2180

[#] Cramer values were generated *a postori* by the Taxon software and are a measure of the usefulness of individual attributes for sorting strains into clusters. A value of one means that the attribute was consistent across all groups. Lesser values indicate that the attribute was less consistent within groups.

Green Manures Pot Trial 1 - Results from first sowing.

Treatment		Tubers ≥ 5cc				Soil properties at harvest			Cellulase	
Green Manure	Inoculum	% scab area	log (% scab area)	Number per plant	Mean tuber weight, (g)	pH	E.C. (µS/cm)	Kjeldahl Nitrogen %	30 days (at sowing)	70 days (tuber init.)
Green Manures										
None	-	2.7	0.49	1.4	20.3	6.8	65	0.24	15.0	16.8
Broad Bean	-	1.5	0.33	1.6	24.8	6.6	141	0.25	37.2	20.5
Triticale	-	3.1	0.50	1.7	25.5	6.6	104	0.24	37.2	32.3
Standard Error		0.40	0.042	0.15	1.39	0.03	8.4	0.002	2.37	1.54
n		6	6	6	6	6	6	6	6	6
p		0.034	0.024	0.460	0.043	0.001	>0.001	0.002	>0.001	>0.001
lsd(0.05) or n.s.		**1.23**	**0.129**	**n.s.**	**4.28**	**0.10**	**25.8**	**0.006**	**7.31**	**4.75**
Inoculum										
-	None	2.7	0.47	1.5	25.9	6.7	104	0.24	30.5	22.9
-	Strain #23	2.2	0.40	1.6	21.2	6.7	103	0.24	29.1	23.6
Standard Error		0.33	0.034	0.12	1.13	0.03	6.8	0.002	1.94	1.26
n		9	9	9	9	9	9	9	9	9
p		0.269	0.186	0.534	0.014	0.271	0.894	0.43	0.635	0.688
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**3.49**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Interaction										
None	None	2.9	0.51	1.3	21.9	6.9	60	0.23	15.0	14.2
None	Strain #23	2.5	0.47	1.6	18.7	6.8	70	0.24	15.0	19.5
Broad Bean	None	1.7	0.38	1.6	28.8	6.6	149	0.25	37.6	21.6
Broad Bean	Strain #23	1.3	0.28	1.5	20.9	6.6	133	0.25	36.8	19.4
Triticale	None	3.5	0.53	1.7	26.9	6.6	103	0.24	38.8	32.7
Triticale	Strain #23	2.7	0.47	1.8	24.1	6.6	105	0.24	35.7	31.8
Standard Error		0.57	0.059	0.21	1.96	0.05	11.8	0.003	3.35	2.18
n		3	3	3	3	3	3	3	3	3
p		0.904	0.900	0.642	0.384	0.921	0.552	0.344	0.895	0.228
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Notes

¥ Nine plants per pot

If p≥ 0.05 then least significant difference is shown, otherwise **n.s** indicates not significant.

E.C. = electrical conductivity

Cellulase measured as reduction in vicosity of a carboxymethyl cellulose solution at 25°C.

Green Manures Pot Trial 1 - Soil elemental analysis at harvest following first sowing

Treatment		Elements in parts per million								
Green Manure	Inoculum	P	K	Ca	Mg	Mn	Zn	Cu	B	Combined Millieqivalents K, Ca and Mg per 100g
Green Manures										
None	-	25	485	2500	495	455	3.3	3.7	2.1	17.8
Broad Bean	-	26	630	2605	520	445	3.5	3.7	1.8	18.9
Triticale	-	27	695	2580	520	450	3.4	3.7	1.8	19.0
Standard Error		0.9	27.4	21.8	2.9	7.1	0.10	0.15	0.13	0.16
n		2	2	2	2	2	2	2	2	2
p		0.534	0.026	0.084	0.013	0.65	0.465	0.963	0.294	0.022
lsd(0.05) or n.s.		**n.s.**	**123.24**	**n.s.**	**13.0**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**0.7**
Inoculum										
-	None	25	580	2553	513	447	3.4	3.6	2.0	18.5
-	Strain #23	26	627	2570	510	453	3.4	3.8	1.7	18.6
Standard Error		0.7	62.9	34.7	8.5	5.3	0.09	0.08	0.11	0.39
n		3	3	3	3	3	3	3	3	3
p		0.349	0.628	0.751	0.795	0.422	0.621	0.145	0.193	0.776
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Notes

If p≥ 0.05 then least significant difference is shown, otherwise n.s indicates not significant.

Green Manures Pot Trial 1 - Soil plate counts at harvest following first sowing.

Treatment		GAT agar				CMC agar		
Green Manure	Inoculum	Actinomycetes + melanin	Actinomycetes - melanin	Other bacteria	Fungi	Actinomycetes	Other bacteria	Fungi
Green Manures								
None	-	11.2	2.3	13.3	0.5	29.3	22.3	0.3
Broad Bean	-	20.2	4.5	32.8	0.7	42.2	25.0	0.2
Triticale	-	13.2	3.8	21.0	1.8	42.0	39.5	0.3
Standard Error		4.48	0.79	5.60	0.81	7.74	5.15	0.25
n		6	6	6	6	6	6	6
p		0.361	0.180	0.083	0.466	0.430	0.076	0.868
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Inoculum								
-	None	15.7	3.2	26.6	1.2	36.2	30.3	0.4
-	Strain #23	14.0	3.9	18.2	0.8	39.4	27.6	0.1
Standard Error		3.66	0.64	4.57	0.66	6.32	4.20	0.21
n		9	9	9	9	9	9	9
p		0.753	0.478	0.222	0.641	0.725	0.649	0.279
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Interaction								
None	None	13.0	2.0	13.7	0.7	26.7	18.0	0.7
None	Strain #23	9.3	2.7	13.0	0.3	32.0	26.7	0.0
Broad Bean	None	21.3	3.7	38.3	1.3	45.7	27.7	0.3
Broad Bean	Strain #23	19.0	5.3	27.3	0.0	38.7	22.3	0.0
Triticale	None	12.7	4.0	27.7	1.7	36.3	45.3	0.3
Triticale	Strain #23	13.7	3.7	14.3	2.0	47.7	33.7	0.3
Standard Error		6.34	1.11	7.92	1.14	10.94	7.28	0.36
n		3	3	3	3	3	3	3
p		0.931	0.677	0.703	0.767	0.702	0.389	0.661
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Notes

Colony forming units x 10^4 per gram of air dried soil.
n.s. indicates not significant at $p \leq 0.05$.

Green Manures Pot Trial 1 - Results from second sowing.

Treatment		Tubers >2.0g						
Green Manure	Inoculum	Number of Lesions	Lesions per Tuber Weight	Lesions per Tuber	Tuber Weight per Plant (g)	Tuber Number per Plant	E.C. (µS/cm)	pH
Green manures								
None	-	50.0	0.69	2.7	7.8	2.0	78	6.7
Broad Bean	-	55.7	0.62	2.7	9.9	2.2	106	6.6
Triticale	-	76.0	0.65	3.0	13.4	2.8	112	6.7
Standard Error		11.17	0.111	0.43	0.81	0.18	8.4	0.04
n		6	6	6	6	6	6	6
p		0.2625	0.8984	0.8652	0.0011	0.0143	0.032	0.074
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**2.49**	**0.54**	**25.9**	**n.s.**
Inoculum								
-	None	56.1	0.63	2.8	9.6	2.1	96	6.7
-	Isolate 23	65.0	0.67	2.8	11.2	2.6	102	6.6
Standard Error		9.12	0.091	0.35	0.66	0.14	6.9	0.03
n		9	9	9	9	9	9	9
p		0.5038	0.7316	0.9847	0.1078	0.0323	0.502	0.454
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**0.45**	**n.s.**	**n.s.**
Interaction								
None	None	42.3	0.58	2.3	7.3	1.8	75	6.8
Broad Bean	None	40.7	0.51	2.5	9.1	1.9	100	6.6
Triticale	None	85.3	0.80	3.6	12.2	2.6	112	6.7
None	Isolate 23	57.7	0.80	3.1	8.2	2.1	82	6.7
Broad Bean	Isolate 23	70.7	0.72	3.0	10.7	2.6	112	6.6
Triticale	Isolate 23	66.7	0.50	2.3	14.7	3.0	113	6.6
Standard Error		15.80	0.157	0.61	1.14	0.25	11.9	0.05
n		3	3	3	3	3	3	3
p		0.3216	0.2128	0.2257	0.7721	0.6936	0.909	0.821
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Notes

¥ Nine plants per pot

E.C. = electrical conductivity

If p≥ 0.05 then least significant difference is shown, otherwise n.s indicates not significant.

Green Manures Pot Trial 2 - Results from first sowing.

Treatment		Tuber yield		Soil properties at harvest			
Green Manure	Inoculum (strain)	Tuber Weight per Plant #	Tuber Number per Plant #	pH	E.C. (µS/cm)	FDA Hydrol. §	Cellulase ¥
Green Manures							
None	-	15.2	2.8	7.8	159	0.030	13.1
Lupin	-	16.5	2.4	7.7	224	0.041	29.4
Rye	-	17.7	3.0	7.8	214	0.045	30.9
Mustard	-	17.0	2.8	7.8	219	0.038	23.9
Standard error		0.82	0.20	0.02	12.9	0.0031	1.41
n		15	15	3	3	3	3
p		0.180	0.186	0.219	0.024	0.060	<0.001
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**42.1**	**n.s.**	**4.61**
Inoculum							
-	none	17.3	2.7	7.8	211	0.041	25.2
-	2/2-5	16.8	2.9	7.8	193	0.039	23.5
-	25/2	15.6	2.6	7.8	209	0.036	24.3
Standard error		0.71	0.17	0.03	17.8	0.0037	4.17
n		20	20	4	4	4	4
p		0.248	0.440	1.000	0.743	0.642	0.962
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Interactions							
None	none	15.2	2.6	7.8	169	0.035	13.8
None	2/2-5	14.6	2.9	7.8	144	0.031	14.4
None	25/2	15.7	2.7	7.8	165	0.026	11.2
Lupin	none	17.8	1.9	7.7	257	0.042	29.7
Lupin	2/2-5	16.0	2.5	7.7	193	0.045	25.3
Lupin	25/2	15.7	2.7	7.8	223	0.035	33.1
Rye	none	17.0	2.9	7.8	200	0.040	30.7
Rye	2/2-5	19.0	3.2	7.8	235	0.047	30.9
Rye	25/2	17.1	2.9	7.8	207	0.047	31.0
Mustard	none	19.3	3.2	7.8	216	0.046	26.5
Mustard	2/2-5	17.5	3.1	7.8	199	0.032	23.5
Mustard	25/2	14.1	2.1	7.7	241	0.035	21.8
Standard error		1.42	0.35	*	*	*	*
n		5	5	1	1	1	1
p		0.345	0.288	*	*	*	*
lsd(0.05) or n.s.		**n.s.**	**n.s.**	*	*	*	*

Notes

Three plants per pot

E.C. = electrical conductivity

§ FDA hydrolysis measured as absorbance at 490nm.

¥ Cellulase measured as reduction in vicosity of a carboxymethyl cellulose solution at 25°C.

If p≥ 0.05 then least significant difference is shown, otherwise n.s indicates not significant.

* Could not be determined with pooled samples, (n=1)

Green Manures Pot Trial 2 - Results from second sowing.

Treatment		Scab	Tuber Weight	Soil properties at harvest			
Green Manure	Inoculum	Lesions per plant#	per Plant #	pH	E.C.	FDA Hydrol. §	Cellulase ¥
Green Manures							
None	-	0.20	14.3	7.8	151	0.040	23.0
Lupin	-	0.09	17.6	7.7	187	0.038	30.5
Rye	-	0.22	15.9	7.8	178	0.042	31.9
Mustard	-	0.18	12.2	7.8	175	0.043	27.7
Standard error		0.084	0.86	0.02	8.3	0.0028	0.78954
n		15	15	15	15	9	15
p		0.694	0.000	0.059	0.022	0.648	0.00
lsd(0.05) or n.s.		**n.s.**	**2.43**	**n.s.**	**23.5**	**n.s.**	**2.239**
Inoculum							
-	none	0.08	13.9	7.8	184	0.036	27.8
-	2/2-5	0.12	16.0	7.8	163	0.042	28.9
-	25/2	0.32	15.1	7.8	171	0.044	28.1
Standard error		0.073	0.74	0.01	7.2	0.0024	0.68
n		20	20	20	20	12	20
p		0.058	0.150	0.075	0.113	0.076	0.533
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Interactions							
None	none	0.07	14.693333	7.8	156	0.037	22.9
None	2/2-5	0.07	14.446667	7.8	144	0.039	23.7
None	25/2	0.47	13.786667	7.8	151	0.043	22.4
Lupin	none	0.07	16.56	7.7	206	0.033	29.7
Lupin	2/2-5	0.00	18.633333	7.7	169	0.041	30.6
Lupin	25/2	0.20	17.713333	7.8	186	0.040	31.1
Rye	none	0.13	14.173333	7.7	198	0.037	31.5
Rye	2/2-5	0.27	17.606667	7.7	173	0.043	32.5
Rye	25/2	0.27	15.866667	7.8	163	0.046	31.7
Mustard	none	0.07	10.22	7.8	177	0.036	27.1
Mustard	2/2-5	0.13	13.293333	7.8	165	0.044	28.6
Mustard	25/2	0.33	13.006667	7.8	184	0.048	27.4
Standard error		0.145	1.49	0.03	14.4	0.004895	1.36753
n		5	5	5	5	3	5
p		0.877	0.864	0.29104	0.782	0.992	0.992
lsd(0.05) or n.s.		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Notes

Three plants per pot

E.C. = electrical conductivity

§ FDA hydrolysis measured as absorbance at 490nm.

¥ Cellulase measured as reduction in vicosity of a carboxymethyl cellulose solution at 25°C.

If p≥ 0.05 then least significant difference is shown, otherwise n.s indicates not significant.

Effect of *S. scabies* and S. *violaceusniger* on growth of green manure plants.

Pathogen Inoculum	Dry-weight Measurments (grams) Lupin Vegetation	Lupin Roots *	Lupin Veg/Root ratio	Rye Vegetation	Rye Roots *	Rye Veg/Root ratio	Mustard# Vegetation
Control (nil)	12.4	9.1	1.64	14.1	6.5	2.35	21.1
***S. scabies* strain 1/2-3**	9.9	2.9	4.97	13.0	5.9	2.26	16.5
***S. violaceusniger* strain 46/1A-1**	13.0	4.9	3.64	11.9	5.1	2.33	17.0
Standard error	1.93	1.75	0.872	1.78	1.03	0.228	1.33
n	5	5	5	5	5	5	5
p	0.489	0.074	0.056	0.683	0.612	0.959	0.058
lsd(0.005) or n.s. §	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

* Root weight measurements are approximate due to difficulty in collecting fine roots.

Mustard root weight was not determined due to difficulty in seperating fine roots from potting mix.

§ If p≥ 0.05 then least significant difference is shown, otherwise **n.s** indicates not significant.

Field Sites - Potato yield and scab data

1) Springfield Site

Treatment/Block	Number of tubers per plant	Tuber Weight per Plant (kg)	Mean tuber weight (g)	Scab Lesions per plant	Scab Lesions per tuber
Treatments					
Oat	10.3	2.231	225.2	0.2	0.01
Lupin	11.0	2.389	237.2	1.9	0.15
Ryegrass	10.2	2.389	259.9	1.8	0.14
Fallow	10.7	2.011	211.8	0.8	0.08
Canola	10.2	2.241	227.2	33.4	3.74
Standard error	1.05	0.1887	21.55	6.02	0.592
n	12	12	12	12	12
p	0.973	0.612	0.602	0.001	<0.001
lsd(0.05) or n.s.	**n.s.**	**n.s.**	**n.s.**	**17.1**	**1.68**
Within Blocks					
Block A	10.1	1.983	216.9	0.9	0.08
Block B	10.9	2.492	249.3	10.6	0.95
Block C	10.4	2.282	230.6	11.5	1.43
Standard error	0.814	0.1462	16.69	4.66	0.459
n	20	20	20	20	20
p	0.807	0.057	0.395	0.219	0.119
lsd(0.05) or n.s.	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Interaction					
Oat Block A	11.75	2.259	199.321	0.5	0.04
Oat Block B	12	2.58175	218.149	0	0
Oat Block C	7	1.85175	257.999	0	0
Lupin Block A	9.75	2.25775	258.966	2.8	0.26
Lupin Block B	11.25	2.7375	271.63	3	0.19
Lupin Block C	12	2.172	181.03	0	0
Ryegrass Block A	6.5	1.8395	302.021	0	0
Ryegrass Block B	12	2.4905	218.767	0	0
Ryegrass Block C	12	2.8378	258.912	5.5	0.42
Fallow Block A	13	1.732	135.146	0	0
Fallow Block B	7.5	2.082	303.719	0.3	0.02
Fallow Block C	11.5	2.21875	196.48	2.3	0.21
Canola Block A	9.5	1.8255	189.162	1.3	0.12
Canola Block B	11.5	2.5675	234.157	49.5	4.55
Canola Block C	9.5	2.32975	258.379	49.5	6.54
Standard error	1.82	0.3269	37.33	10.43	1.026
n	4	4	4	4	4
p	0.054	0.566	0.046	0.213	0.064
lsd(0.05) or n.s.	**n.s.**	**n.s.**	**105.8**	**n.s.**	**n.s.**

Notes

If p≥ 0.05 then least significant difference is shown, otherwise n.s indicates not significant.

Field Sites - Potato yield and scab data

2) Deloraine Site

Treatment/Block	Number of tubers per plant	Tuber Weight per Plant (kg)	Mean tuber weight (g)	Scab Lesions per plant	Scab Lesions per tuber
Treatments					
Oat	11.3	3.629	353	12	1.03
Lupin	9.2	3.089	367	4.8	0.49
Ryegrass	12.5	3.646	316	2.5	0.23
Fallow	9.8	3.396	374	3.9	0.59
Standard error	1.16	0.2413	28.7	2.25	0.237
n	12	12	12	12	12
p	0.186	0.338	0.497	0.024	0.131
lsd(0.05) or n.s.	**n.s.**	**n.s.**	**n.s.**	**6.41**	**n.s.**
Within Blocks					
Block A	9.7	3.345	376	5.6	0.69
Block B	11.1	3.52	347	2.7	0.24
Block C	11.2	3.454	335	9.1	0.84
Standard error	1	0.2089	24.8	1.95	0.205
n	16	16	16	16	16
p	0.495	0.837	0.499	0.078	0.111
lsd(0.05) or n.s.	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Interaction					
Oat Block A	10.3	3.809	376	13.2	1.21
Oat Block B	7.8	3.246	430	0.5	0.06
Oat Block C	15.8	3.83	253	22.3	1.84
Lupin Block A	8.5	2.97	378	2.8	0.34
Lupin Block B	11	3.375	326	6.8	0.51
Lupin Block C	8	2.924	398	4.8	0.62
Ryegrass Block A	11.8	3.555	347	0	0
Ryegrass Block B	14.3	3.85	286	0	0
Ryegrass Block C	11.5	3.535	316	7.7	0.69
Fallow Block A	8.3	3.048	401	6.3	1.19
Fallow Block B	11.5	3.612	346	3.5	0.37
Fallow Block C	9.5	3.529	374	2	0.21
Standard error	2	0.4179	49.7	3.9	0.41
n	4	4	4	4	4
p	0.131	0.801	0.287	0.055	0.143
lsd(0.05) or n.s.	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Notes

If $p \geq 0.05$ then least significant difference is shown, otherwise n.s indicates not significant.

Field Sites - Soil enzyme activity, pH and conductivity

1) Springfield Site

Date	FDA Hydrolysis Oat	Lupin	Ryegrass	Fallow	Canola	S.E.	p	lsd(0.05)
11/6/1997	0.13	0.16	0.16	0.16	0.13	0.011	0.197	n.s.
25/8/1997	0.18	0.25	0.17	0.12	0.16	0.010	0.000	**0.032**
5/11/1997	0.16	0.18	0.21	0.20	0.14	0.013	0.020	**0.040**
3/2/1998	0.16	0.19	0.18	0.19	0.15	0.009	0.011	**0.027**

Date	Cellulase Oat	Lupin	Ryegrass	Fallow	Canola	S.E.	p	lsd(0.05)
11/6/1997	24.4	21.2	25.2	19.5	21.9	1.59	0.208	n.s.
25/8/1997	36.4	38.9	36.6	38.6	36.3	1.13	0.399	n.s.
5/11/1997	33.2	32.4	28.7	28.8	31.5	2.27	0.544	n.s.
3/2/1998	41.2	40.0	40.1	38.2	33.5	2.19	0.174	n.s.

Date	Soil pH Oat	Lupin	Ryegrass	Fallow	Canola	S.E.	p	lsd(0.05)
11/6/1997	5.0	5.0	4.9	4.9	5.0	0.06	0.711	n.s.
25/8/1997	4.7	4.7	4.7	4.8	4.6	0.08	0.756	n.s.
5/11/1997	4.7	4.8	4.6	4.7	4.8	0.08	0.690	n.s.
3/2/1998	4.8	4.5	4.7	4.6	4.9	0.10	0.090	n.s.

Date	Electrical Conductivity Oat	Lupin	Ryegrass	Fallow	Canola	S.E.	p	lsd(0.05)
11/6/1997	70	61	54	67	66	7.2	0.096	n.s.
25/8/1997	109	180	129	150	205	23.2	0.051	n.s.
5/11/1997	142	157	110	117	168	12.8	0.039	**40.3**
3/2/1998	132	254	163	195	148	27.0	0.063	n.s.

2) Deloraine Site

Date	FDA Hydrolysis Oat	Lupin	Ryegrass	Fallow	S.E.	p	lsd(0.05)
19/6/1997	0.09	0.09	0.06	0.08	0.006	0.014	**0.018**
26/8/1997	0.11	0.10	0.06	0.08	0.006	0.002	**0.021**
5/11/1997	0.10	0.09	0.10	0.10	0.007	0.496	n.s.
4/2/1998	0.08	0.08	0.08	0.08	0.004	0.433	n.s.

Date	Cellulase Oat	Lupin	Ryegrass	Fallow	S.E.	p	lsd(0.05)
19/6/1997	17.1	14.9	15.1	15.4	1.76	0.814	n.s.
26/8/1997	27.9	23.9	26.0	23.7	3.26	0.781	n.s.
5/11/1997	23.3	18.2	21.0	19.1	1.62	0.192	n.s.
4/2/1998	17.8	11.2	15.6	13.3	2.82	0.427	n.s.

Date	Soil pH Oat	Lupin	Ryegrass	Fallow	S.E.	p	lsd(0.05)
19/6/1997	5.5	5.4	5.4	5.5	0.07	0.486	n.s.
26/8/1997	5.5	5.5	5.5	5.5	0.07	0.732	n.s.
5/11/1997	5.5	5.6	5.6	5.6	0.06	0.659	n.s.
4/2/1998	5.6	5.7	5.7	5.7	0.10	0.681	n.s.

Date	Electrical Conductivity Oat	Lupin	Ryegrass	Fallow	S.E.	p	lsd(0.05)
19/6/1997	100	71	107	103	12.0	0.097	n.s.
26/8/1997	105	85	90	95	4.1	0.118	n.s.
5/11/1997	151	107	117	112	10.0	0.054	n.s.
4/2/1998	192	129	122	144	8.3	0.001	**27.2**

Notes

Figures show the mean of three replicates.

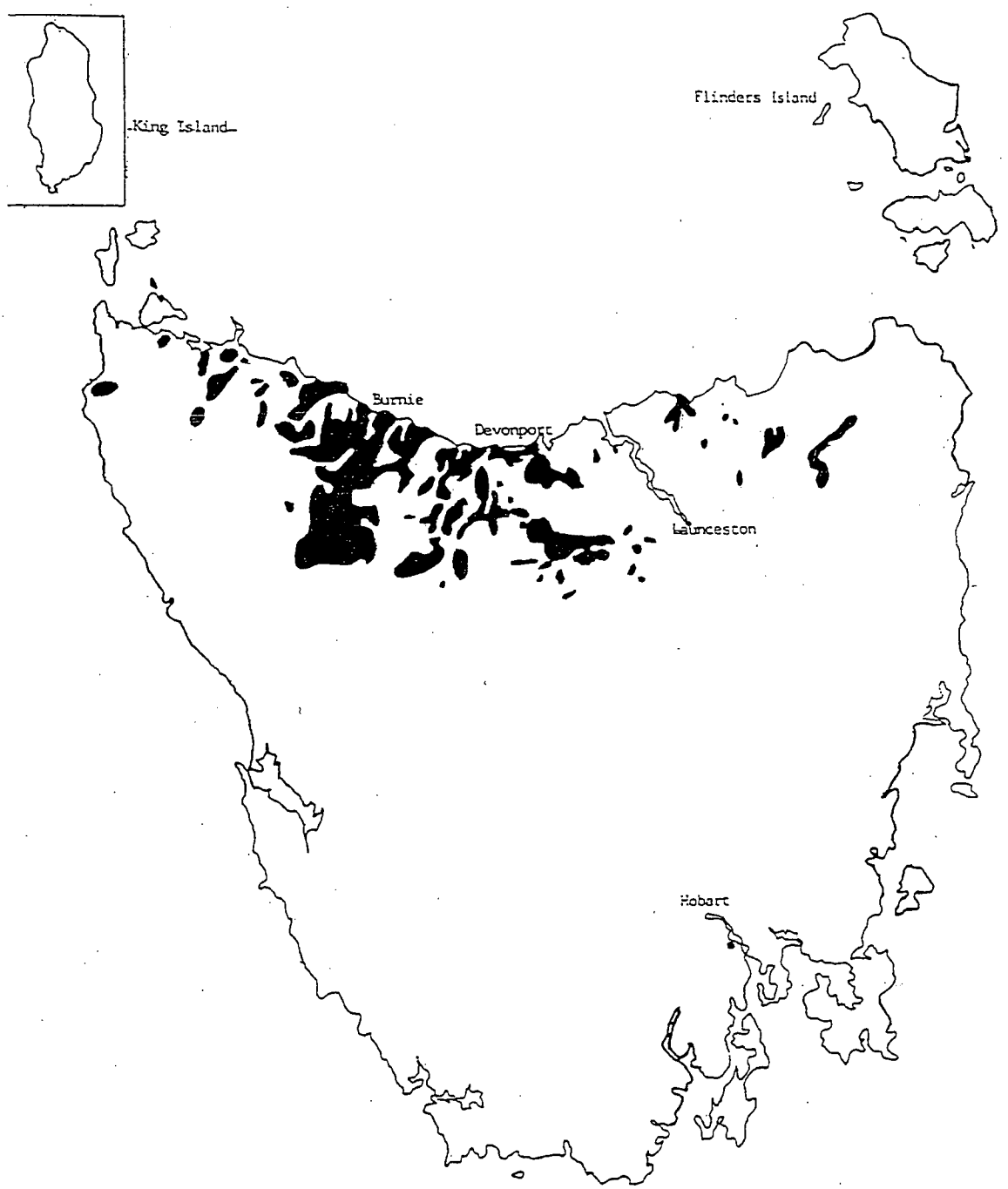
If p≥ 0.05 then least significant difference is shown, otherwise n.s indicates not significant.

FDA hydrolysis was measured as absorbance at 490nm.

Cellulase was measured as reduction in vicosity of a carboxymethyl cellulose solution at 25°C.

Appendix 3.1

Map of krasnozem soils in Tasmania (Source: Nichols and Dimmock, 1965.)



Appendix 3.2

Soil chemical and scab index data from the 1994 DPIWE scab survey.

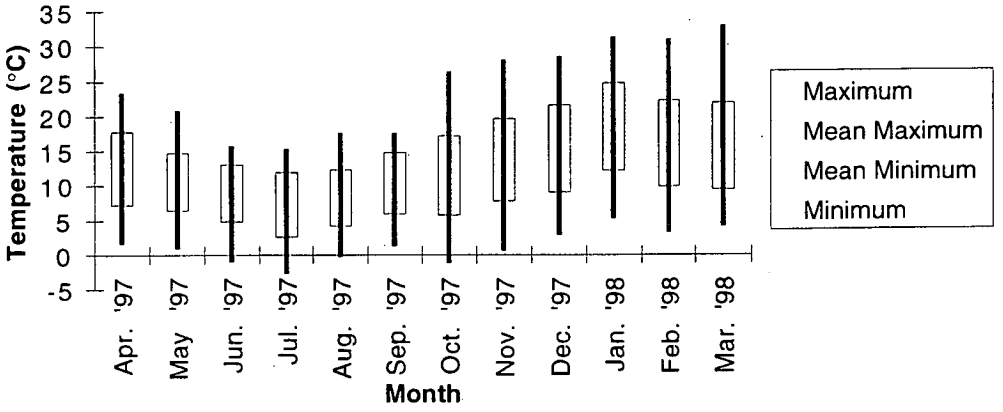
1994 Scab Survey Data

Sample Number	Scab Index	Soil properties											Milliequivalents of exchangeable cations/100g						
		LOI%	pH	EC µS/cm	P ppm	K ppm	Ca ppm	Mg ppm	Mn ppm	Zn ppm	Cu ppm	B ppm	K	Ca	Mg	K+Ca	K+Mg	Ca+Mg	K+Ca+Mg
1	50	17.5	7	85	32	420	3240	290	5	0.6	0.1	1.9	1.07	16.17	2.39	17.24	3.46	18.55	19.63
2	0	22.4	5.5	165	11	250	1750	110	8.8	2.3	0.2	2.5	0.64	8.73	0.91	9.37	1.54	9.64	10.28
3	0	17.2	6	210	35	590	2430	480	18	1.3	0.2	3.1	1.51	12.13	3.95	13.64	5.46	16.08	17.59
4	0	25.4	6	125	46	200	1840	380	24	1.6	0.2	1.7	0.51	9.18	3.13	9.69	3.64	12.31	12.82
5	304	10.3	6.4	95	38	310	1770	360	11	1.2	0.2	1.4	0.79	8.83	2.96	9.63	3.76	11.80	12.59
6	16	19	5.6	180	11	250	1340	160	38	0.8	0.5	2.1	0.64	6.69	1.32	7.33	1.96	8.00	8.64
7	0	21	5.9	160	14	160	1830	200	35	1	0.4	1.5	0.41	9.13	1.65	9.54	2.06	10.78	11.19
8	12	20.8	5.1	305	58	470	950	170	26	1	0.6	2.2	1.20	4.74	1.40	5.94	2.60	6.14	7.34
9	0	25.6	5	265	21	190	1210	110	18	1.1	0.5	2.7	0.49	6.04	0.91	6.52	1.39	6.94	7.43
10	89	21.9	5.7	200	11	180	1520	300	28	1.9	0.8	3.5	0.46	7.58	2.47	8.05	2.93	10.05	10.51
11	93	17.5	5.9	135	21	900	1900	160	23	1.3	0.3	2.3	2.30	9.48	1.32	11.78	3.62	10.80	13.10
12	6	6	6	225	45	310	1120	170	10	1	0.1	1	0.79	5.59	1.40	6.38	2.19	6.99	7.78
13	17	21.2	6.1	150	17	370	2690	330	30	1.8	0.3	2.1	0.95	13.42	2.72	14.37	3.66	16.14	17.09
14	0	26.6	5.3	140	9	360	1050	200	29	1.2	0.4	3.6	0.92	5.24	1.65	6.16	2.57	6.89	7.81
15	90	19.4	6.8	130	23	130	2220	250	1.7	0.6	0.1	2.3	0.33	11.08	2.06	11.41	2.39	13.14	13.47
16	0	18.6	5.9	185	25	320	2460	270	5	0.6	0.1	2.2	0.82	12.28	2.22	13.09	3.04	14.50	15.32
19	37.5	15.4	6	135	11	160	1360	330	15	0.8	0.1	2.1	0.41	6.79	2.72	7.20	3.13	9.50	9.91
22	75.5	20.3	5.6	145	15	530	1950	210	22	1.3	0.3	2.7	1.36	9.73	1.73	11.09	3.08	11.46	12.81
25	132	18.7	5.6	180	30	430	1520	140	19	0.9	0.3	2.6	1.10	7.58	1.15	8.68	2.25	8.74	9.84
28	0	26.5	5	190	18	220	1120	170	32	1.1	0.2	3.5	0.56	5.59	1.40	6.15	1.96	6.99	7.55
29	0	21.5	5.4	140	23	290	1410	320	31	1	0.3	3.3	0.74	7.04	2.63	7.78	3.38	9.67	10.41
30	0	27.8	5.2	270	13	340	900	290	8	0.8	0.5	4.5	0.87	4.49	2.39	5.36	3.26	6.88	7.75
31	5	23.7	5	230	22	310	870	300	23	1.1	0.4	4.6	0.79	4.34	2.47	5.13	3.26	6.81	7.60
32	1	18.5	5.4	155	11	410	1360	190	21	0.7	0.2	3	1.05	6.79	1.56	7.84	2.61	8.35	9.40
33	2.3	20.5	5.9	150	20	640	2310	240	18	1.7	0.3	2.4	1.64	11.53	1.98	13.16	3.61	13.50	15.14
34	880	20.3	5.9	90	22	280	2240	320	16	1.2	0.1	1.7	0.72	11.18	2.63	11.89	3.35	13.81	14.53
35	5	19.1	5.4	260	17	120	1710	60	26	1	0.3	2.9	0.31	8.53	0.49	8.84	0.80	9.03	9.33
36	27	18.8	5.2	160	13	170	1790	310	46	1.5	0.2	3	0.43	8.93	2.55	9.37	2.99	11.48	11.92
37	5	15.5	5.7	115	28	330	1400	190	12	0.6	0.2	3	0.84	6.99	1.56	7.83	2.41	8.55	9.39
38	0	14.7	6.4	95	36	380	2360	210	16	0.8	0.2	2	0.97	11.78	1.73	12.75	2.70	13.50	14.48
39	0	13.2	5.9	60	12	190	1390	200	13	1.3	0.2	2.4	0.49	6.94	1.65	7.42	2.13	8.58	9.07

Appendix 3.3

Monthly temperature and rainfall data for Scottsdale (5 km north of the Springfield site) for the period April 1997 to March 1998. (Source: Australian Bureau of Meteorology data for Station Number 91219.)

Temperature Data for Scottsdale -April1997 to March 1998



Rainfall Data for Scottsdale - April 1997 to March 1998